DOPAMINE

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Volume 21

DOPAMINE

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Foreword for the Handbook of Chemical Neuroanatomy

By Paul Greengard

For a period of about 40 years, from 1930 until 1970, a vigorous debate raged within the neuroscience community as to the mechanisms underlying what we today call fast synaptic transmission. There were two schools of thought. The electrical school argued that as the nerve impulse reached the axon terminal, the wave of depolarization caused a change in the electric field across the postsynaptic plasma membrane resulting in an excitatory or an inhibitory post synaptic potential. The chemical school argued that the wave of depolarization at the nerve terminal, associated with the arrival of the nerve impulse, caused an influx of calcium through voltage-sensitive calcium channels and resulted in the fusion of neurotransmitter-containing vesicles with the presynaptic membrane, the ensuing release of neurotransmitter and the activation of hypothetical receptors in the postsynaptic membrane. The debate ended in a resounding victory for the chemical school. It is now clear that over 99% of all fast synaptic communication between nerve cells in the brain is chemical in nature. We also know that the neurotransmitter, released from the presynaptic terminal, activates the ligand-operated ion channels initiating a physiological response in the target cell.

The role, and even the existence, of slow synaptic transmission was even more hotly debated. Some of the strongest evidence in support of the slow chemical transmission came from studies of the neurotransmitter/neuromodulator dopamine. The studies by Arvid Carlsson and his colleagues, and by other investigators who followed shortly thereafter, provided compelling evidence that Parkinson's disease was attributable to the degeneration of the dopaminergic neurons with the resultant loss of regulation by dopamine of target cells in the neostriatum. The fact that levodopa treatment could abolish the symptoms of Parkinsonism, both in the experimental animals and patients, finally convinced the neuroscience community of the important role this biogenic amine plays in communication between nerve cells. Studies of the mechanisms by which slow-acting neurotransmitters produce their effects on their target cells have revealed unexpectedly complex signaling pathways. As a result of the complexity of the mechanisms underlying slow synaptic transmission, compared to fast synaptic transmission, the literature on slow signaling pathways has become the dominant literature in the field. The vital and complex roles that dopamine and other biogenic amines play in the physiology and the pathophysiology of the brain have become subjects of increasingly intense scientific investigation. The literature on dopamine alone is now so vast that it is almost impossible for any one scientist to follow it. This volume of The Handbook of Chemical Neuroanatomy, coming after more than 20 years since the initial volume in this series, will be of great help for anyone trying to cope with this ever-burgeoning literature.

Preface

In this, the 21st volume of the Handbook of Chemical Neuroanatomy, we are revisiting the topic of Dopamine systems of the forebrain, first covered 20 years ago in the 2nd volume in the series on Classical Neurotransmitters of the CNS. In the earlier volume, the anatomy of dopamine, noradrenaline and adrenaline systems has been described in detail. The chapter on the dopamine pathways of the forebrain, by Björklund and Lindvall giving a detailed mapping of the ascending dopamine system, provided a classic account that remains little changed even after two decades, other than in the fine detail. By contrast, what has changed dramatically in the intervening years has been the very many developments in our understanding of the functional organization of all forebrain transmitter systems, not just dopamine. Our understanding of dopamine systems in particular, has been profoundly influenced by the advent of new techniques in molecular biology, neurogenetics, single cell and membrane physiology, and clinical neurology, neuropsychiatry and brain imaging in vivo. In this volume, we seek to provide a systematic overview of the major recent developments in our understanding of the chemical neuroanatomy of the forebrain dopamine systems from a functional perspective. Nowadays, it requires a whole volume dedicated just to dopamine in order to provide comprehensive reviews of the key developments for this one neurotransmitter.

After a generous foreword by Paul Greengard, in the first chapter, Bentivoglio and Morelli provide a systematic overview of the morphological and neurochemical background on the organisation of the midbrain dopamine systems and their ascending forebrain projections and receptors, to provide the anatomical foundation and overall context for the more specific themes in each of the subsequent chapters. Horne et al. then consider the opportunities of transgenic technologies to understand the roles of different classes of dopamine receptors both in mediating functional processes, such as reward and in regulating neuronal plasticity and sprouting. The molecular focus on receptors is then carried forward by Hervé and Girault, in reviewing the alternative mechanisms of signal transduction by G proteins and cAMP at the different classes of dopamine receptors. The physiological consequences of such interactions are then considered by Wickens and Arbuthnott, discussing the functional implications of the spatial and temporal specificity of the dopamine signal.

The dopamine system has been one of the major foci of attention in the behavioral neurosciences throughout this period, because of the pharmacological and the toxic tools available for its selective manipulation and the resulting dramatic influences on key dimensions of motor, motivational and cognitive functions. Consequently the following three chapters by Dunnett, Di Chiara, and Robbins in turn review the recent developments in each of these domains of behavioral function. Next, the chapter by Lookingland and Moore provides a separate consideration to the hypothalamic dopamine systems and the very different endocrine functions also subserved by dopamine neurotransmission. Finally, Hurd and Hall consider the uniquely human disturbances in psychiatric function, associated with changes in dopamine transmission, from the perspective provided by recent developments in imaging, both in vivo and postmortem.

The editors wish to thank all the authors who have responded so willingly to contribute their time and expertise in preparing their individual chapters to a consistently high standard. We hope that you find the resulting synthesis a welcome addition to the literature by providing systematic critical reviews and a lasting reference source of contemporary developments in the functional neuroanatomy of the forebrain dopamine systems.

STEPHEN DUNNETT (Cardiff, Wales, UK) May 2004
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CHAPTER I

The organization and circuits of mesencephalic dopaminergic neurons and the distribution of dopamine receptors in the brain

MARINA BENTIVOGLIO AND MICAELA MORELLI

ABSTRACT

The organization of the main dopaminergic cell groups in the brain, located in the ventral mesencephalic tegmentum, and the circuits in which they are inserted are reviewed here, with emphasis on rodents. Subdivisions based on cytoarchitecture (substantia nigra, ventral tegmental area and related nuclei, retrorubral field), dopaminergic phenotype (A8, A9 and A10 cell groups) and organization in dorsal and ventral tiers are discussed and compared. Dendritic release and gap junctional protein expression, interactions with glial cells, molecular and cellular features of the chemical repertoire of midbrain dopaminergic neurons and their main inputs are also reviewed. An account is given on basal ganglia circuits, including the organization of the direct, indirect and hyperdirect pathways of information processing and dopamine modulation of these pathways. Data on the dopaminergic innervation of limbic structures, including the extended amygdala, and the distribution and laminar organization of dopaminergic fibers in the cerebral cortex are summarized. The last part of the chapter focuses on the distribution of dopamine receptor subtypes and their relative densities in different brain structures. For each of the D_1, D_2, D_3, D_4 and $D_{1B/5}$ receptors, an overview and distributional maps are provided, followed by data on their localization in the rat basal ganglia, cerebral cortex and limbic system, and a comparison with findings obtained in the human and nonhuman primate brain. This chapter thus presents an overview, at the molecular, cellular and systems levels, of central dopaminergic circuits involved in state-setting modulatory systems, generation and integration of motor behavior, cognitive functions and reward mechanisms.

KEY WORDS: Basal ganglia; substantia nigra; ventral tegmental area; striatum; globus pallidus; subthalamic nucleus; limbic system.

1. INTRODUCTION

The organization, cellular features and molecular signature, as well as the functional correlates of the circuits which utilize dopamine (DA) as neurotransmitter represent one of

the most fertile fields of investigation in neuroscience. Interest in these circuits and their regulation has been and still is stimulated by their involvement in neurological and psychiatric diseases, besides their role in motor and cognitive functions, and in the motivational aspects of behavior in the normal brain. Thus, 40 years after the pioneering description of the mesencephalic dopaminergic cell groups by Dahlström and Fuxe (1964), and 20 years after the classical chapters by Björklund and Lindvall (1984) and Hökfelt et al. (1984a) in the Handbook of Chemical Neuroanatomy, the central dopaminergic systems are still in the forefront of neuroscience.

The overviews of Björklund and Lindvall (1984) and Hökfelt et al. (1984a) appeared 20 years after the report of Dahlström and Fuxe (1964) of monoamine-containing cell groups in the central nervous system by means of the Falck-Hillarp histofluorescence technique (see Section 1.1). Novel technical approaches, developed in the last two decades, have been applied to the study of dopaminergic neurons. Knowledge of these cells and circuits has thus been enriched by findings obtained with immunohistochemistry, molecular biology techniques, the use of transgenic mice and conditional mutants for the study of the role of molecules and as animal models of diseases, functional anatomy including the mapping of neurons activated by given stimuli through the induction of immediate early genes, electrophysiology including chronic recording, sophisticated behavioral analysis, imaging techniques including functional neuroimaging and imaging of receptors. In addition, the last two decades have witnessed a rapid development of studies on DA receptors, leading also to the discovery of DA receptor subtypes. The anatomical organization of dopaminergic pathways has thus been animated by novel functional correlates and enriched by molecules as protagonists and co-actors, regulated by complex mechanisms and interactions. Altogether, these studies have not only added new knowledge, but have also led to new conceptual frameworks on the healthy and pathological functioning of dopaminergic circuits at the molecular, cellular and system levels.

In the first chapter of this volume, we will review the organization of the main dopaminergic cell groups in the brain, which are located in the ventral tegmentum of the mesencephalon, and the circuits in which they are inserted. The organization of hypothalamic dopaminergic cell groups and circuits is reviewed in the chapter by Lookingland and Moore in this volume. We will also focus on the distribution of DA receptors in the brain, to summarize current information on the brain geography of these key effectors of DA action. Signal transduction mechanisms of DA receptors are dealt with in the chapter of Hervé and Girault, and interactions in the striatum at the receptor level in the chapter of Wickens and Arbuthnott.

An account of the dopaminergic systems in the human forebrain is given by Hurd and Hall in this volume, and a chapter on these systems in the brain of primates has already appeared in the Handbook of Chemical Neuroanatomy (Lewis and Sesack, 1997). The present chapter will therefore refer mainly to rodents. Data on dopaminergic cell groups and circuits in other subprimates and in primates will be mentioned, whenever useful for comparison and discussion. Some emphasis will be given instead to the distribution of DA receptors in the primate brain as compared to the rat, in order to provide an overview of the distribution of DA receptor subtypes.

As far as rodents are concerned, it should be noted that the anatomy of mesencephalic dopaminergic systems, in terms of both projections and neurochemical features, has been studied mainly in the rat, and the chapters by Björklund and Lindvall (1984) and Hökfelt et al. (1984a) referred to this species. The mouse, however, is becoming increasingly

important in neuroscience because of its status as an animal model for gene manipulation. In addition, at variance with the rat, in which the selective neurotoxin 6-hydroxydopamine is still the main tool used to induce lesions of the dopaminergic system, DA-containing neurons in mice are sensitive to 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) toxicity (Heikkila et al., 1984). The mouse can, therefore, also provide a rodent model of lesions which characterize Parkinson's disease in humans. A comparison between the organization of the mesencephalic dopaminergic system of the rat and the mouse will, therefore, be discussed whenever data are available.

To place information in the context of an itinerary of knowledge, an overview will first be given of the debates and the methodological developments which led to the identification of central dopaminergic cells and to the elucidation of neuronal networks in which DA exerts its action.

1.1. THE OLD AND THE RECENT TORMENTED HISTORY OF THE MESENCEPHALIC DOPAMINERGIC CELL GROUPS AND THEIR PROJECTIONS

The substantia nigra (SN) was observed in the human brain as a collection of pigmented cells lying dorsal to the cerebral peduncle by Vicq d'Azir, who described it in 1786 as 'locus niger crurum cerebri', and soon after by Sömmerring (1788) whose name was linked to this structure (see, for example, Fig. 1). The SN was then readily identified by pioneers in neuroscience in the midbrain tegmentum ventral to the red nucleus of human adults and during development (Fig. 1) as a cell mass, sandwiched between the huge cerebral peduncles and the medial lemniscus (Meynert, 1888; Mingazzini, 1888; Mirto, 1896; Sano, 1910; Edinger, 1911; Castaldi, 1923). However, the projections of the SN, and more generally those of the ventral midbrain tegmentum, turned out to be very difficult to identify.

The existence of the nigrostriatal pathway was predicted in the neuropathological literature (Von Monakow, 1895; Holmes, 1901) on the basis of retrograde degeneration of SN cells following large telencephalic lesions that involved the cerebral cortex and the striatum. Subsequent studies reported cell loss in the pars compacta of the SN (SNc) after lesions limited to the striatum (reviewed by Hattori, 1993). However, anterograde tracing studies, based on silver impregnation of degenerating fibers, first with the Marchi technique (Marchi and Algeri, 1886) and later with the Nauta technique (Nauta and Gygax, 1951), failed to demonstrate fibers reaching the striatum from the SN.

On the other hand, degeneration of the SN following striatal lesions was ascribed to a transneuronal effect, so that prominent neuroanatomists questioned the existence of the nigrostriatal pathway. For example, Mettler stated in 1970: 'I believe that, at the present time, most neuroanatomists agree that the nigra projects to the pallidum'. Even neuroanatomists determined to verify the nigral output could not find an indication of nigrostriatal fibers in the rat (but could not find evidence of nigropallidal fibers either) with the Nauta technique, and stated that 'if such a pathway does exist, it must be refractory to the Nauta method ... or the terminals may be too fine to be resolved by the light microscope' (Faull and Carman, 1968). However, as it will be outlined, evidence of the dopaminergic nigrostriatal fibers had already been obtained in the mid-1960s. The anatomical confirmation was obtained with the sensitive silver impregnation protocol introduced by Fink and Heimer (1967). Using this technique, in 1970, Moore provided the

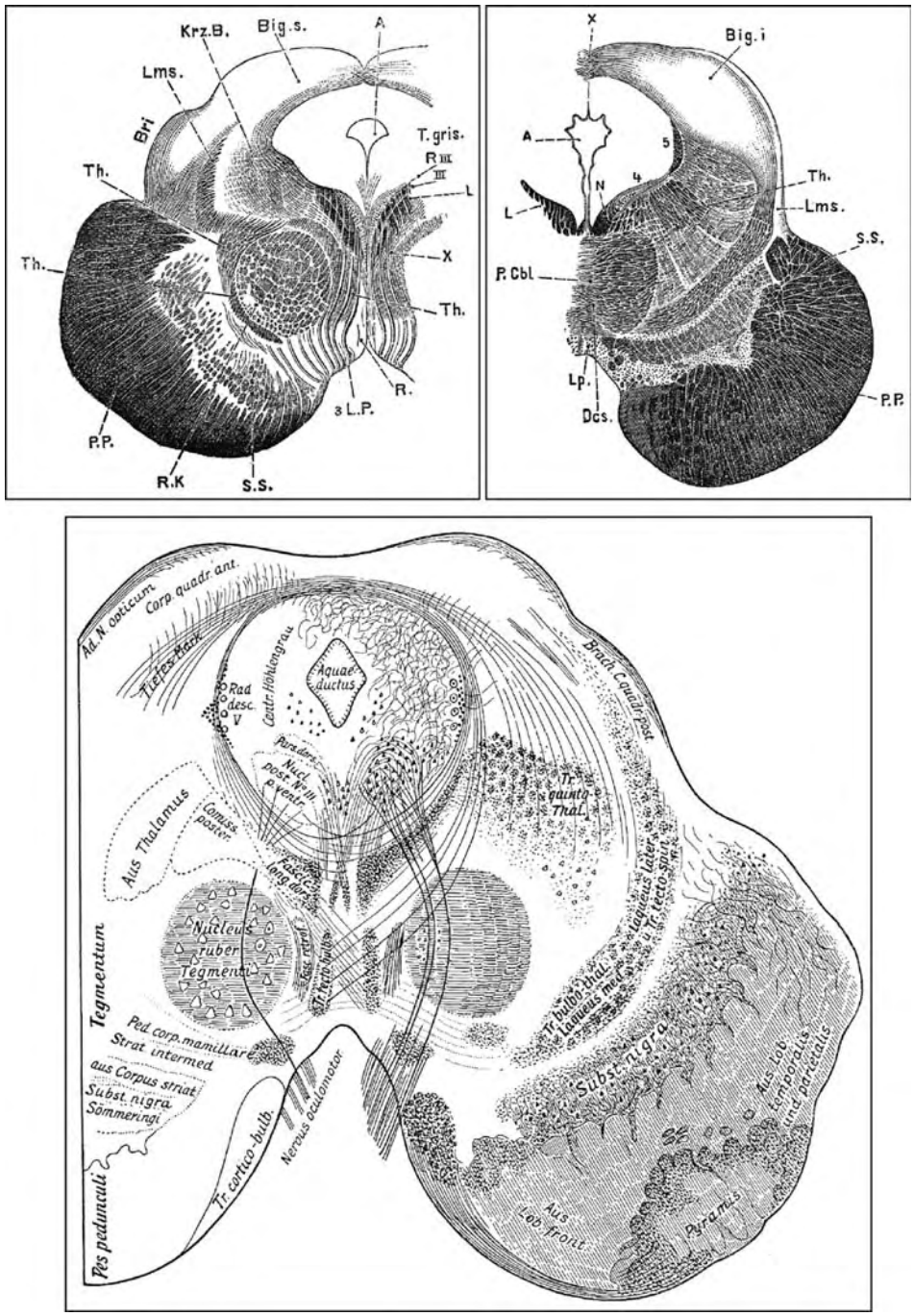

Fig. 1. **Top:** Transverse sections through the human mesencephalon, as drawn by Theodor Meynert from preparations stained with 'gold and potassium chloride'. Abbreviations (translated from the original legends in French): A, aqueduct; Big.s., superior quadrigeminal tubercle; Bri, geniculate body and its bundles; Dcs, decussation of the superior cerebellar peduncle; Krz.B., bundles of the anterior crossing, the X indicates the crossing; L, posterior longitudinal bundle; Lms, lemniscus after the decussation; Pcbl, superior cerebellar

first demonstration of anterograde degeneration in the striatum of the cat following lesions placed in the ventral midbrain tegmentum.

The identification of cells of the SN as dopaminergic and of the dopaminergic innervation of the striatum through the nigrostriatal tract is recent history, inextricably intertwined with methodological achievements in experimental and chemical neuroanatomy in the 1960s and 1970s, and with discoveries on the histopathology of the midbrain dopaminergic system in Parkinson's disease in the 1960s.

As emphasized by Björklund and Lindvall (1984), Carlsson (1959) proposed that DA could play a key role in motor control in the basal ganglia, and that the DA depletion in the striatum could be the cause of neurological symptoms in Parkinson's disease. Soon after, postmortem findings of the reduced levels of DA in the striatum and SN of the brain of Parkinsonian patients (Ehringer and Hornykiewicz, 1960; Hornykiewicz, 1963) led to the suggestion that a disturbance in the DA-containing nigrostriatal tract could represent the primary cause of neurological alterations in Parkinson's disease (Hornykiewicz, 1966).

These studies were paralleled by the demonstration of central monoaminergic neurons at the light microscopic level, which represents a milestone in the history of the dopaminergic system, and of neuroscience in general. This discovery was achieved by the formaldehyde fluorescence method, also known as the Falck-Hillarp technique, and its modifications (Carlsson et al., 1962; Falck, 1962; Falck et al., 1962), based on the condensation of monoamines with formaldehyde resulting in a fluorescent product. In 1964, Dahlström and Fuxe reported in the rat, the occurrence of catecholamine-containing cell bodies in the midbrain (Fig. 2) and lower brain stem. Lesion of the SN was found to cause a substantial loss of catecholamine fluorescence in the striatum (Andén et al., 1964), with accumulation of fluorescent material in axons of the nigrostriatal bundle (Andén et al., 1965), and loss of DA and its synthetic enzymes in the striatum (see Hattori, 1993). Evidence of a nigrostriatal fiber system originating from dopaminergic midbrain neurons was thus obtained while neuroanatomists were still discussing its existence, and these findings inspired the above-mentioned critical experiment which demonstrated nigral efferents to the striatum (Moore, 1970). Even the more skeptical neuroanatomists were then rapidly convinced of the existence of the nigrostriatal pathway, and stated that 'nigral efferent fibers in the globus pallidus appeared entirely *en passage*' (Carpenter and Peter, 1972).

Studies in experimental and chemical neuroanatomy underwent then, as it frequently happens in scientific research, a sudden acceleration. Retrograde axonal transport was discovered on the basis of the finding that proteins, such as the enzyme horseradish peroxidase (HRP), are retrogradely transported from axon terminals to their parent neuronal cell bodies (Kristensson and Olsson, 1971). The modern era of neuroanatomy

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peduncle; P.P., pes pedunculi; R, raphe; RK, red nucleus; R III, III, root and nucleus of the oculomotor nerve; S.S., intermediate layer (literally: '*stratum intermedium*') with the 'substance of Soemmering'; T.gris., central gray substance; Th, bundles of the optic layer for the tegmentum (literally: '*calotte*'); 3L.P., root of the oculomotor and posterior perforated substance. Reproduced from Meynert (1888). **Bottom:** Drawing made by Ludwig Edinger from sections of the human postnatal brain stained with hematoxylin-eosin. Edinger described in the text that the appearance of the substantia nigra illustrated in the drawing reproduced the features observed in the brain of newborns, and pointed out the 'comb-like' appearance of cells that 'fan-out' due to fibers. Reproduced from Edinger (1911).

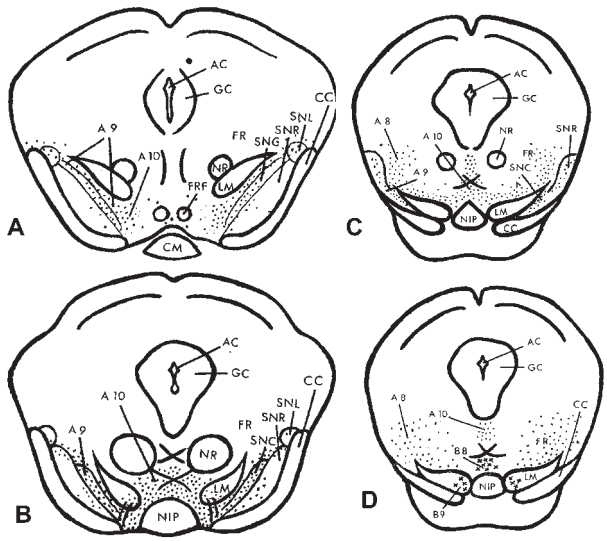

Fig. 2. Schematic representation of the distribution of monoamine-containing cells in the rat midbrain, as illustrated in 1964 by Dahlström and Fuxe in the study in which they first identified these cells and subdivided catecholaminergic cells of the midbrain into A8, A9 and A10 cell groups. The original drawings have here been arranged in rostrocaudal (A–D) order. The original legends specify that 'the catecholamine type cells are indicated with dots and the 5-HT type with crosses'. Abbreviations: AC, aqueduct; A8, A9, A10: catecholamine-containing cell groups; B8, B9: serotonin-containing cell groups; CC, crus cerebri; CM, corpus mammillare; FR, formation reticularis; FRF, fasciculus retroflexus; GC, griseum centralis; LM, lemniscus medialis; NIP, nucleus interpeduncularis; NR, nucleus ruber; SNC, substantia nigra, zona compacta; SNL, substantia nigra, pars lateralis; SNR, substantia nigra, zona reticulata. Reproduced from Dahlström and Fuxe (1964).

started with the introduction of HRP as a retrograde tracer for the study of the origin of neural circuits (La Vail and La Vail, 1972). At the same time, the anterograde axonal transport of tritiated amino acids, whose labeling is revealed by autoradiography, became a tool for the study of termination fields of neural projections (Cowan et al., 1972). With these techniques, not only was the nigrostriatal system definitely ascertained but also it became one of the most studied pathways in the brain.

After the study of La Vail and La Vail (1972) in the visual system, the nigrostriatal projection was the first central pathway investigated with HRP (Kuypers et al., 1974; Nauta et al., 1974), and even became a test pathway for the identification of new retrograde tracers (Kuypers et al., 1977). The availability of tracers (and fluorescent dyes in particular) suited for multiple retrograde labeling allowed the simultaneous study of more than one population of projection neurons and the detection of collateralized pathways. As it will be repeatedly mentioned in this chapter, these techniques were rapidly applied to the study of basal ganglia circuits. New anterograde tracers resulting in high resolution labeling of axons and terminal fields, such as *Phaseolus vulgaris* leucoagglutinin (Gerfen and Sawchenko, 1984), were also introduced in the following years. These tracers proved to be valuable tools for the study of basal ganglia circuits at the light and the electron microscopic levels, including double anterograde tracing techniques (reviewed by Smith et al., 1998).

The technical approaches for the visualization of neuroactive molecules were rapidly progressing in parallel. Geffen et al. (1969) introduced the principle of revealing

monoamines by the immunohistochemical labeling of their synthetic enzymes. The latter study was based on the use of antibodies to dopamine-β-hydroxylase, the enzyme which converts DA to noradrenaline and is present in noradrenergic and adrenergic neurons as well as in cells of the adrenal gland. After working out methodological aspects including formalin fixation of the tissue to be processed with immunohistochemistry (Hökfelt et al., 1973b), Hökfelt and coworkers (1973a) were the first to visualize midbrain dopaminergic neurons with immunohistochemistry using antibodies to aromatic acid decarboxylase, followed by the report of Pickel et al. (1975).

The immunohistochemical revelation of tyrosine hydroxylase (TH), the rate-limiting enzyme of DA synthesis, was a breakthrough in the identification of dopaminergic cells. Such a strategy was adopted by the Swedish investigators (Ljungdahl et al., 1975) in a study which also pioneered double labeling approaches, combining TH immunohistochemistry with retrograde labeling of SNc cells after HRP injection in the striatum (Fig. 3). These findings (Ljungdahl et al., 1975) led to the final confirmation of the dopaminergic nature of the nigrostriatal pathway, and paved the way for the simultaneous investigation of neural circuits and their chemical characterizations (Björklund and Skagerberg, 1979; Sawchenko and Swanson, 1981; Hökfelt et al., 1983; Skirboll et al., 1984), also at the ultrastructural level (see Smith et al., 1998; Sesack, 2003).

Last but not least, altogether, these studies inspired the series of the Handbook of Chemical Neuroanatomy, whose first volume appeared in 1983.

2. THE DOPAMINERGIC NEURONS OF THE VENTRAL MIDBRAIN TEGMENTUM

2.1. CRITERIA OF NOMENCLATURE AND SUBDIVISION

As all the brain regions and systems attract a great deal of attention and effort by the investigators, the nomenclature and subdivisions of the ventral midbrain tegmentum and of the DA-containing neurons distributed in this region have gone through revisions, reflecting new knowledge and deeper insight. This, however, may create some confusion when approaching the topic nowadays, and problems in the use of key words for the electronic search in literature data base, as well as in the comparison among different studies. It is therefore important to outline the different approaches to the subdivision of the midbrain dopaminergic cell groups, and the conceptual homologies and differences between such approaches.

We will deal below with the subdivisions based on three different criteria that reflect the evolution of the theoretical concepts and the technical advances based on: (i) cytoarchitectonic features, (ii) the dopaminergic phenotype of neurons, and (iii) the organization of midbrain dopaminergic neurons into dorsal and ventral tiers. Cytoarchitectonic features are observed with nonspecific cell staining, such as the Nissl staining, routinely used for the study of the nervous tissue. The definition of different catecholamine-containing cell groups in the midbrain was introduced by Dahlström and Fuxe in 1964, when these cells were first observed, and is still widely used in studies referring to DA-containing cells. The subdivision into dorsal and ventral tiers derives from connectivity findings obtained with the axonal transport of tracers, together with data on the spatial arrangement of cell bodies and their processes obtained with the Golgi impregnation and other methods of cellular filling, as well as with chemoarchitectural data

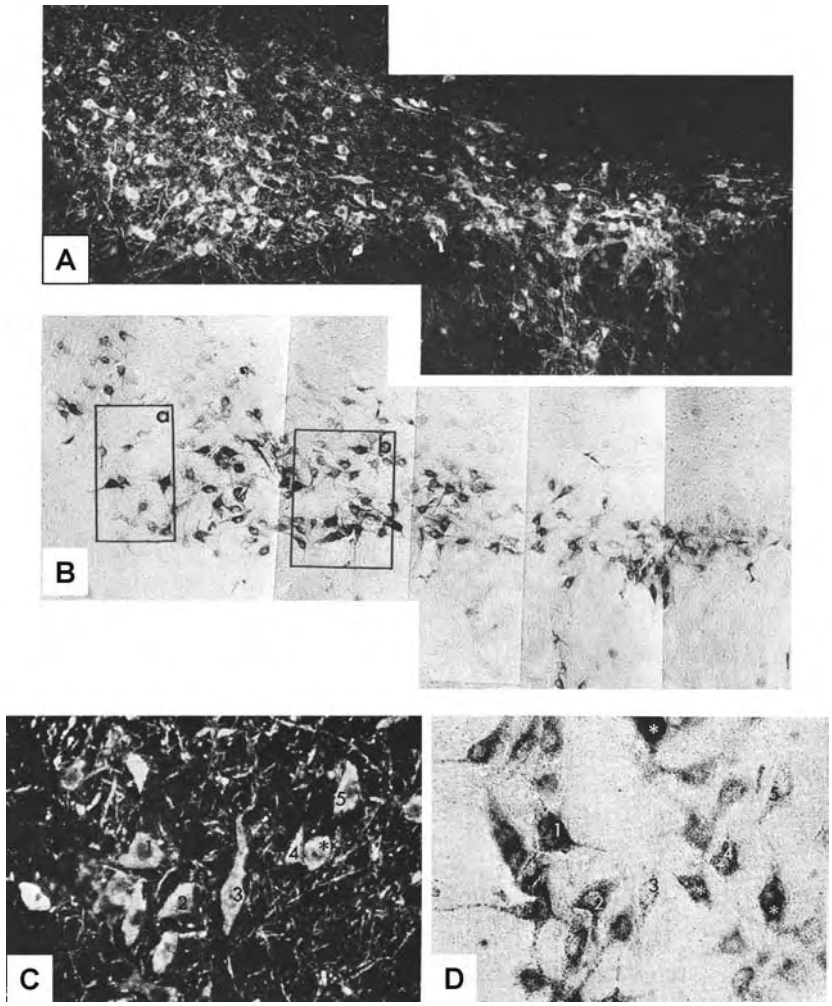

Fig. 3. The plate reproduces illustrations of the first study in which dopaminergic neurons of the substantia nigra were characterized by tyrosine hydroxylase immunopositivity (TH, revealed by immunohistofluorescence in A and C) and simultaneously identified as nigrostriatal neurons through retrograde labeling (B and D are bright-field micrographs of the same fields shown in A and C, respectively, under fluorescence observation). Retrograde labeling was obtained by injection of the tracer horseradish peroxidase (HRP) 'in the head of the caudate nucleus' (as stated in the original legend) of the rat. The combined strategy was based on incubation with antibodies to TH and photography, followed by the histochemical procedure for HRP demonstration. A and B provide low power views, and the original legend states: 'The distribution of TH and HRP positive cells is very similar. Note that in A both cell bodies and cell processes are strongly stained, whereas the HRP reaction is confined mainly to the cell bodies'. The framed areas in B were illustrated at higher magnification, showing in pairs the immunofluorescence and the HRP labeling. In particular, C and D correspond to the framed area indicated with 'b' in the low power view of HRP labeling. The original legend states: 'most cells (1–5) contain both TH and HRP, whereas some cells are only TH positive (black asterisks) and others are only HRP positive (white asterisks)' and specifies that the weak appearance of some HRP-labeled cells in the bright-field micrograph was due to the fact that these cells were slightly out of focus as a consequence of the section thickness. Reproduced from Ljungdahl et al. (1975).

obtained by means of immunohistochemistry and in situ hybridization. The subdivision into dorsal and ventral tiers is relatively new and has rapidly become a classical criterion for the classification of midbrain dopaminergic neurons also in primates (see Haber, 2003). All the three criteria for the subdivision of the mesencephalic dopaminergic cell groups are, however, currently adopted in the literature.

As mentioned earlier, emphasis here will be given to the organization of the mesencephalic DA system in rodents, and the reader is referred to Lewis and Sesack (1997) and Haber (2003) for findings in primates.

2.2. CYTOARCHITECTONIC SUBDIVISIONS AND NEURONAL FEATURES

2.2.1. Midbrain nuclei containing dopaminergic cells

The dopaminergic neurons of the midbrain are distributed in a continuum across a number of anatomical structures (Figs. 2, 4–8). On the basis of cytoarchitectonic features, the main dopaminergic cell groups are located in the SNc, in the ventral tegmental area (VTA) medial to the SN, and in the retrorubral area (RRA), or retrorubral nucleus (as defined in the cat by Berman (1968) and in the rat by Swanson (1982)) which lies caudal and dorsal to the SN.

Additional nuclei which contain dopaminergic cells have been identified in the ventromedial tegmentum of the rat midbrain on the basis of cytoarchitectonic criteria (Phillipson, 1979a). Three of these nuclei are medial: the rostral linear nucleus of the raphe, the caudal linear nucleus of the raphe (also called central linear nucleus, as defined in the cat by Berman (1968); this structure was also denominated nucleus linearis intermedius in the cat by Taber (1961)), and the interfascicular nucleus located just medial to the fasciculus retroflexus. Two other nuclei are more lateral and include the paranigral nucleus and the parabrachial pigmented nucleus. Although the relative prominence of these nuclei varies across species, the parabrachial pigmented nucleus is consistently the largest of these components in the rat, cat and primates, with a relatively high development also of the interfascicular nucleus in the rat (Halliday and Törk, 1986). The DA-containing cells distributed throughout these structures are part of the A10 cell group identified by Dahlström and Fuxe (1964), as determined by cytoarchitectonic criteria combined with glyoxylic acid histofluorescence (Phillipson, 1979a), and as observed with TH immunohistochemistry (Hökfelt et al., 1984a) (Figs. 7 and 8; see Section 2.3). Therefore, although Halliday and Törk (1986) preferred to define this region as ventromedial mesencephalic tegmentum because it is formed by different nuclear entities, the above-mentioned nuclei, and especially the paranigral and parabrachial pigmented nuclei, may be collectively considered part of the VTA (as suggested by Swanson (1982); see Fig. 5).

Figures 4–6 show the cytoarchitectonic subdivisions which contain dopaminergic cells in the ventral midbrain tegmentum, as illustrated in stereotaxic atlases of the rat and mouse brain. These atlases nowadays represent common laboratory tools, especially for young researchers (who may not be necessarily experts in sophisticated neuroanatomical subdivisions and nomenclature). The SN and its different subdivisions (described in Section 2.2.2) are clearly delineated in Figures 4–6. Medially to the SN, the emphasis on the parcellation (or lack of parcellation) into different nuclei varies slightly according to the authors. The VTA is obviously indicated in all atlases, but its extent is rarely delineated, though the boundaries of this region are outlined at rostral levels in Swanson's

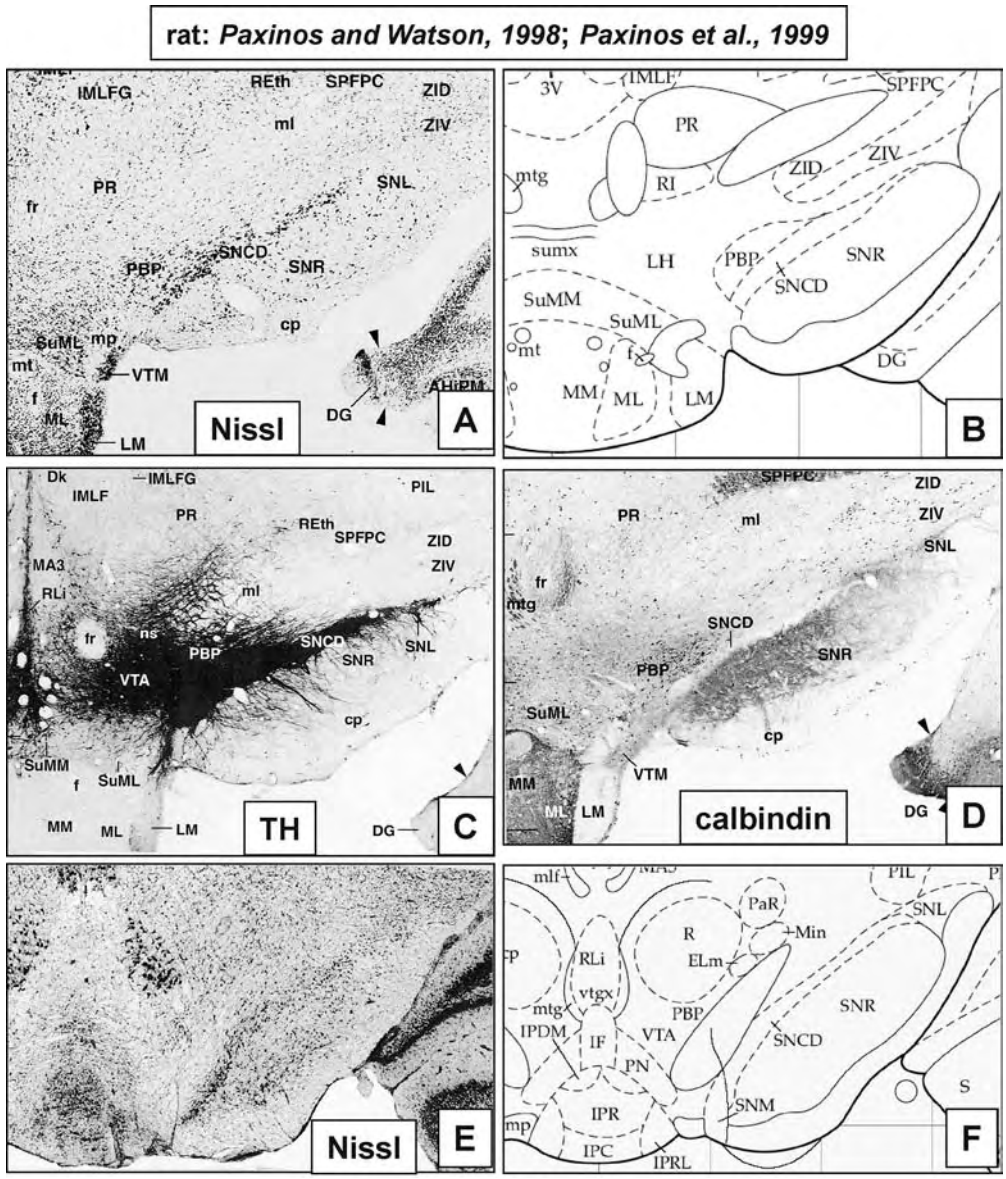

Fig. 4. The ventral midbrain tegmentum as illustrated at rostral (A–D) and middle (E,F) levels in coronal sections through the rat brain in the atlases by Paxinos and coworkers. A,C,D derive from Paxinos et al. (1999); B,E,F from Paxinos and Watson (1998). C and D reproduce sections processed for immunohistochemistry with antibodies to tyrosine hydroxylase (TH) or to the calcium binding protein calbindin. Abbreviations: Cli, central linear nucleus of the raphe; cp, cerebral peduncle; DG, dentate gyrus; DpMe, deep mesencephalic nucleus; dtgx, dorsal tegmental decussation; f, fornix; fr, fasciculus retroflexus; IMLF, interstitial nucleus of the medial longitudinal fasciculus; IPC, interpeduncular nucleus, caudal subnucleus; IPDL, interpeduncular nucleus, dorsolateral; IPDM, interpeduncular nucleus, dorsomedial; IPI, interpeduncular nucleus, intermediate subnucleus; IPL, interpeduncular nucleus, lateral subnucleus; IPR, interpeduncular nucleus, rostral subnucleus; LM, lateral mammillary nucleus; ml, medial lemniscus; ML, medial mammillary nucleus, lateral part; mlf, medial longitudinal fasciculus; MM, medial mammillary nucleus, medial part; mp, mammillary peduncle; mt, mammillothalamic tract; mtg, mammillotegmental tract; PBP, parabrachial pigmented nucleus;

atlas of the rat (1992; Fig. 5A) and in the mouse atlas of Hof et al. (2000; Fig. 6A). The location of the parabrachial pigmented nucleus is indicated (but not delimited) by Paxinos and co-workers in the rat (Paxinos and Watson, 1998) and in the mouse (Franklin and Paxinos, 1997; Paxinos and Franklin, 2001; Fig. 6C). The paranigral nucleus is delineated both in the rat (Fig. 4F) and in the mouse (Fig. 6A; the extent of the paranigral nucleus is also delineated in the atlas of Franklin and Paxinos (1997), but at levels more caudal than that shown in Fig. 6C,D). The sections shown in Figures 4–6 also indicate in the rat and the mouse, the location and boundaries of the midline structures which contain dopaminergic cells: the interfascicular nucleus (Figs. 4E and F, 5C and D, 6) and the raphe nuclei (rostral linear nucleus in Figs. 4E and F, 5C and D, 6; central linear nucleus in Figs. 5C and D, 6).

2.2.2. Substantia nigra

Two main subdivisions have been recognized in the SN since the first detailed studies of this structure (Mingazzini, 1888; Sano, 1910; Cajal, 1911). In particular, Mingazzini (1888), who impregnated human midbrain tissue with the Golgi technique, was so impressed by the appearance of the different portions of the SN that he considered the organization of this structure similar to the layered organization of the cerebral cortex and described the SN neurons as pyramidal cells.

Cajal (1911) stated that 'two zones or cellular bands' were recognizable in the SN in transverse Nissl-stained sections through the midbrain: 'the lower one is large and cell poor, but on the contrary rich in protoplasmic processes [dendrites] and fibers of passage; the upper or marginal one is narrow and richer in nerve cells'. Applying the Golgi impregnation to the SN of different animal species, Cajal (1911) clearly described a 'general tendence' towards a 'perpendicular' orientation of dendrites (Fig. 9), which, as will be emphasized below, turned out much later to represent a major feature of SN dopaminergic cells. By the way, to offer to the junior and senior researchers a consolation for the hassle of literature update at present times, it is worth noting that Cajal (1911), probably unaware of Mingazzini's study which had appeared in 1888, mentioned that the SN had first been impregnated with the Golgi staining by Mirto in 1896.

The two main subdivisions of the SN are the SNc, characterized by densely packed neurons (as the Latin adjective 'compacta' indicates), and the pars reticulata (SNr) characterized by sparser cells, enmeshed in fibers (which are the termination of the striatonigral pathway) as in a net (as the Latin adjective 'reticulata' indicates) (Figs. 4A,B,E,F; 5 and 6). A third portion, the pars lateralis (SNl), is formed by a small elliptical mass of neurons in the rostral and the dorsolateral portion of the SN (Figs. 4A,E,F and 6). The SNl has many features in common with the other two subdivisions,

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PP, peripeduncular nucleus; PR, prerubral field; Reth, retroethmoid nucleus; RMC, red nucleus, magnocellular; RPC, red nucleus, parvocellular; scp, superior cerebellar peduncle; SNC, substantia nigra, compact part; SNL, substantia nigra, lateral part; SNR, substantia nigra, reticular part; SPFPC, subparafascicular thalamic nucleus, parvocellular part; SuML, supramammillary nucleus, lateral part; VTA, ventral tegmental area; VTM, ventral tuberomammillary nucleus; ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; 3, oculomotor nucleus; 3n, oculomotor nerve or its root. Reproduced with permission from Paxinos and Watson (1998) and Paxinos et al. (1999).

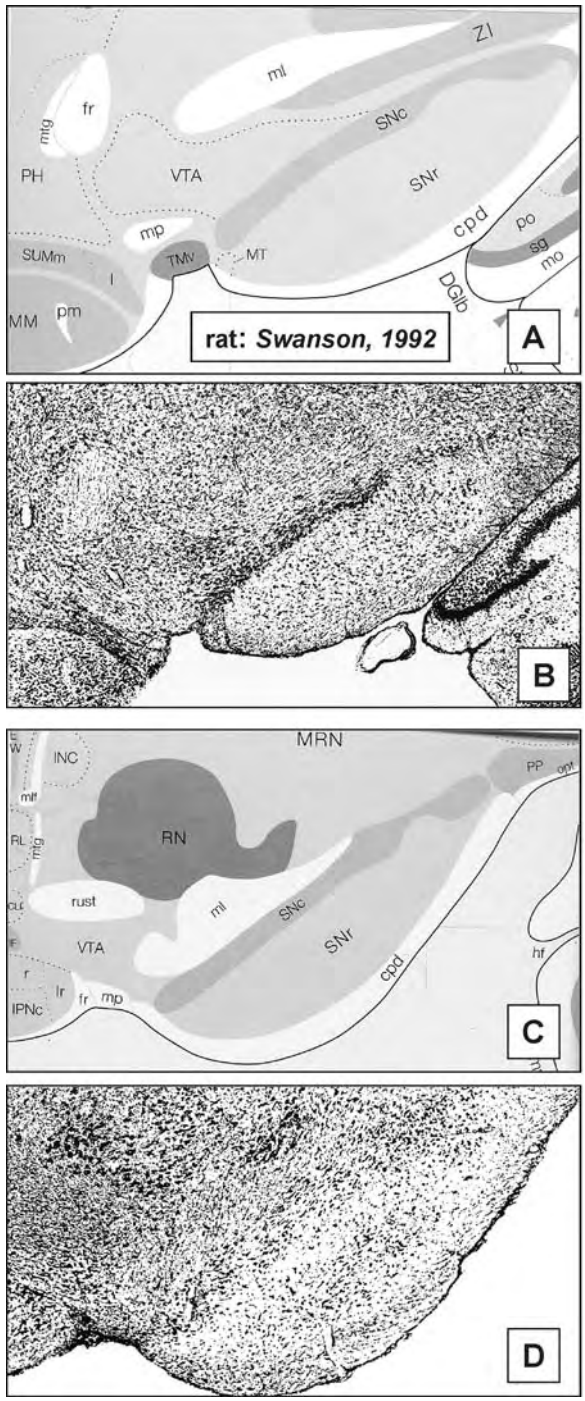

Fig. 5. The plate illustrates the ventral midbrain tegmentum as illustrated in the atlas of the rat brain of Swanson (1992), at levels approximately equivalent to those shown in Fig. 4. B and D are images of Nissl-stained sections. Abbreviations: CLI, central linear nucleus of the raphe; cpd, cerebral peduncle; DGlb, dentate gyrus, lateral blade; EW, Edinger-Westphal nucleus; fr, fasciculus retroflexus; hf, hippocampal fixure; IF, interfascicular

and contains mostly medium-sized cells of various shapes resembling those of the SNc neurons. DA-containing neurons are concentrated in the SNc and are also found in the SNl (Figs. 4A, 7, 8). The SNl shares the projections of the SNc to the striatum and the amygdala (see further, Sections 5.2 and 7.2) but has also some distinct features of connectivity. In particular, nondopaminergic neurons of the SNl project to the inferior colliculus (see the review by Fallon and Loughlin (1995)).

According to the study of Poirier et al. (1983), in the rat the SN of either side has about 22,400 neurons, and 44% belong to the SNc, whereas in the cat, the SN has about 38,400 neurons (58% of which belong to the SNc), and the proportion of SNc cells increases in primates (about 73,500 neurons in the SN, 85% of which are located in the SNc). With some unavoidable variation, these numbers are roughly in agreement with the quantitative evaluations of the DA-containing cells identified with TH immunoreactivity (see Section 2.3).

The cytoarchitectural organization of the SN has been described with Nissl staining (Hanaway et al., 1970; Poirier et al., 1983; Halliday and Törk, 1986)). The cell types and their processes have been identified by Golgi impregnation (Juraska et al., 1977; Phillipson, 1979b) and intracellular filling (Tepper et al., 1987). Neuronal cell bodies in the SNc have various shapes (ovoid, polygonal, or fusiform), and sizes. Halliday and Törk (1986) reported that the perikaryal diameter of the SN neurons ranges from 6 to 33 μm in the rat, and SN neurons are relatively larger in primates (with diameters ranging from 11 to 43 μm in the SNc of the macaque monkey, and from 14 to 50 μm in the human SNc).

In both the SN and the VTA, dopaminergic cell bodies show with Nissl staining a marked basophilia, whereas nondopaminergic neurons, intermingled with dopaminergic ones especially in the VTA, are more lightly stained (Domesick et al., 1983). These light microscopic features correspond, at the electron microscopic level, to ultrastructural characteristics distinctive of dopaminergic neurons, whose cytoplasm appeared filled with regularly arranged rows of rough endoplasmic reticulum cisternae and free ribosomes, indicating a high protein synthesis activity (Domesick et al., 1983).

In the Golgi preparations of the rat midbrain tegmentum (Juraska et al., 1977; Phillipson, 1979b), neurons of the SNc were seen to emit long dendrites which branched infrequently (exhibiting features that overall matched the Cajal's drawings shown in Fig. 9). The dendritic field was found to be oriented mediolaterally in the dorsal part of the SNc, whereas ventrally placed SNc neurons, exhibiting the morphology of inverted pyramids with the base lying dorsally, were seen to emit a long apical dendrite oriented in a dorsoventral direction and extending into the SNr. These findings fit well with the subdivision of midbrain dopaminergic cells into dorsal and ventral tiers (see Section 2.4).

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nucleus of the raphe; INC, interstitial nucleus of Cajal; IPNc, interpeduncular nucleus, central subnucleus; IPNlr, interpeduncular nucleus, lateral subnucleus, rostral part; IPNr, interpeduncular nucleus, rostral subnucleus; ml, medial lemniscus; mlf, medial longitudinal fasciculus; MM, medial mammillary nucleus; mo, molecular layer of dentate gyrus, lateral blade; mp, mammillary peduncle; MRN, mesencephalic reticular nucleus; MT, medial terminal nucleus of the accessory optic tract; mtg, mammillotegmental tract; opt, optic tract; PH, posterior hypothalamic nucleus; pm, principal mammillary tract; po, polymorph layer of dentate gyrus, lateral blade; PP, peripeduncular nucleus; RL, rostral linear nucleus of the raphe; RN, red nucleus; rust, rubrospinal tract; sg, granule cells layer of dentate gyrus, medial blade; SNc, substantia nigra, compact part; SNr, substantia nigra, reticular part; so, stratum oriens of CA1 field; SUMl, supramammillary nucleus, lateral part; SUMm, supramammillary nucleus, medial part; SUMl, supramammillary nucleus, lateral part; TMv, tuberomammillary nucleus, ventral part; VTA, ventral tegmental area; ZI, zona incerta.

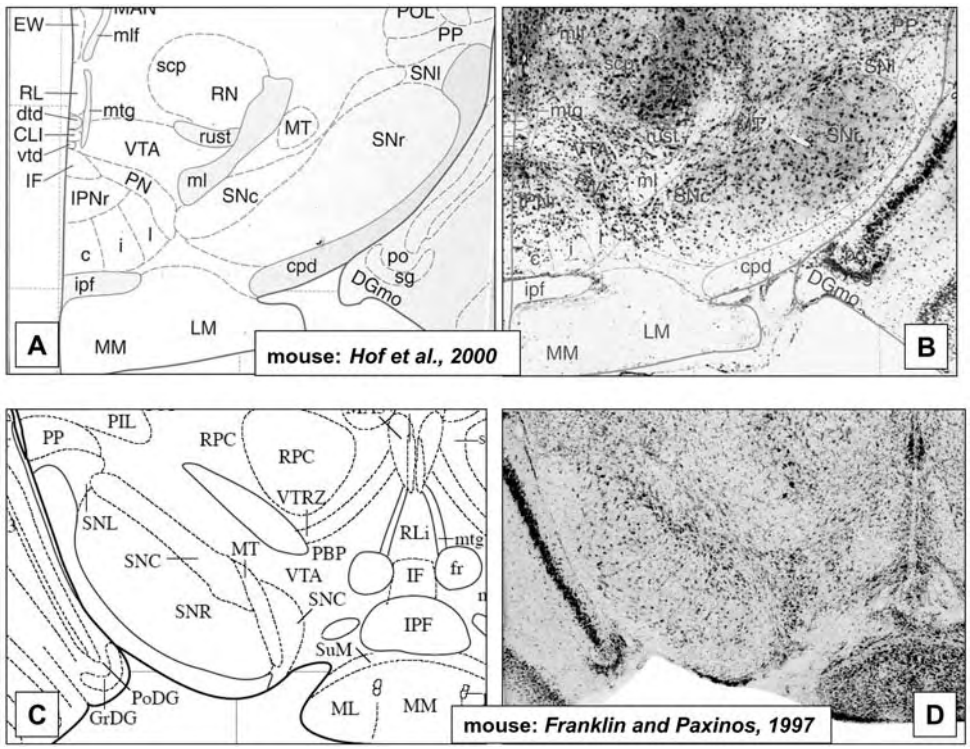

Fig. 6. The plate illustrates a section through the rostral level of the ventral midbrain tegmentum as presented in two different atlases of the mouse brain, to show nuclear subdivisions delineated by different authors, and for a comparison between the mouse and the rat (shown in Figs. 4 and 5). *Abbreviations in A, B*: CLI, central linear nucleus of the raphe; cpd, cerebral peduncle; DGmo, dentate gyrus, molecular layer; dtd, dorsal tegmental decussation; EW, Edinger-Westphal nucleus; IF, interfascicular nucleus; ipf, interpeduncular fossa; IPNc, interpeduncular nucleus, caudal part; IPNi, interpeduncular nucleus, intermediate part; IPNl, interpeduncular nucleus, lateral part; IPNr, interpeduncular nucleus, rostral part; LM, lateral mammillary nucleus; ml, medial lemniscus; mlf, medial longitudinal fasciculus; MM, medial mammillary nucleus; MT, medial terminal nucleus of the accessory optic tract; mtg, mammillotegmental tract; PN, paranigral nucleus; po, polymorphic layer; POL, posterior limitans nucleus of the thalamus; PP, peripeduncular nucleus; RL, rostral linear nucleus of the raphe; RN, red nucleus; rust, rubrospinal tract; scp, superior cerebellar peduncle; sg, granule cell layer; SNc, substantia nigra, compact part; SNl, substantia nigra, lateral part; SNr, substantia nigra, reticular part; VTA, ventral tegmental area; vtd, ventral tegmental decussation. *Abbreviations in C, D*: fr, fasciculus retroflexus; GrDG, granular layer of the dentate gyrus; IF, interfascicular nucleus; IPF, interpeduncular fossa; ML, medial mammillary nucleus, lateral; MM, medial mammillary nucleus, medial; MT, medial terminal nucleus of the accessory optic tract; mtg, mammillotegmental tract; PBP, parabranchial pigmented nucleus; PIL, posterior intralaminar thalamic nucleus; PoDG, polymorph layer of the dentate gyrus; PP, peripeduncular nucleus; RLi, rostral linear nucleus of the raphe; RPC, red nucleus, parvocellular; SNC, substantia nigra, compact part; SNL, substantia nigra, lateral part; SNR, substantia nigra, reticular part; SuM, supramammillary nucleus; VTA, ventral tegmental area; VTRZ, visual tegmental relay zone.

On the other hand, neurons in the most ventral part of the SNr were seen to give off dendrites oriented parallel to the cerebral peduncle.

Intracellular HRP injections (Tepper et al., 1987) also visualized cell bodies that emitted 3–6 primary dendrites, some of which extended ventrally into the SNr, bearing spine-like appendages or other extrusions, especially in their distal portions. With intracellular HRP

Fig. 7. The figure corresponds to the first extensive and detailed study by Hökfelt and coworkers (1976) based on immunoreactivity to tyrosine hydroxylase to visualize dopaminergic neurons of the rat ventral midbrain tegmentum (in cryostat-cut sections). The plate was obtained by mounting several different fluorescence micrographs to provide a complete overview of the region. The original legend indicates that the arrow points to numerous TH-positive cell bodies surrounding the roots of the oculomotor nerve, which also extended into the zona compacta (zc) and zona lateralis (zl) of the substantia nigra. The legend also states that in the zona reticulata (zr) 'a few groups of fluorescent cell bodies are observed, but mainly dendrites from the compacta cells are running in this area'. The double arrow points to varicose axons in the midline, the arrowheads to a small densely packed group of TH-positive neurons in the midline; the crossed arrow points to TH-positive cell bodies within the ventromedial part of the medial lemniscus. The A9 and A10 cell groups were named after Dahlström and Fuxe (1964). CC, crus cerebri; ip, interpeduncular nucleus. Reproduced with permission from Hökfelt et al. (1976).

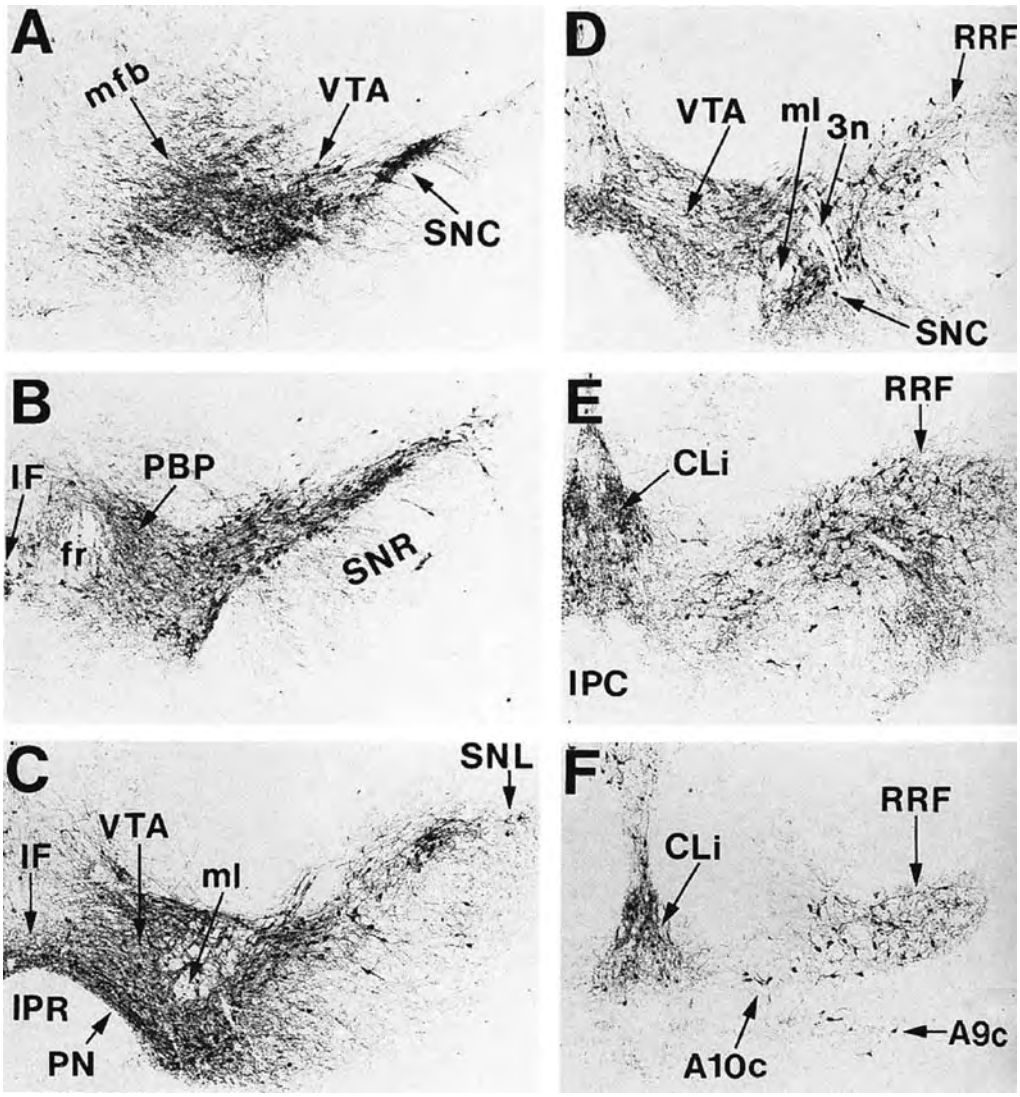

Fig. 8. The plate illustrates the distribution of dopaminergic cells in the mouse, as shown by tyrosine hydroxylase immunoreactivity in coronal sections through the midbrain of the C57BL/6 mouse. Abbreviations: A9c, caudal part of the A9 cell group; A10c, caudal part of the A10 cell group; CLi, central linear nucleus; fr, fasciculus retroflexus; IF, interfascicular nucleus; IPC, caudal interpeduncular nucleus; IPR, rostral interpeduncular nucleus; mfb, medial forebrain bundle; ml, medial lemniscus; PBP, nucleus parabrachialis pigmentosus; PN, nucleus paranigralis; RRF, retrorubral field; SNC, substantia nigra, pars compacta; SNL, substantia nigra, pars lateralis; SNR, substantia nigra, pars reticulata; VTA, ventral tegmental area; 3n, third nerve. Reproduced with permission from Nelson et al. (1996).

filling, SN axons revealed dense collateral arborizations, branching not only within the dendritic field of the parent cell but also in more distant regions of the SN. A peculiar feature observed with the intracellular HRP injections in the axons of the SNc and SNr in the rat, and also in the cat SNr (Karabelas and Purpura, 1980), was represented by the finding that some intrinsic collaterals were seen to terminate on dendrites of the parent

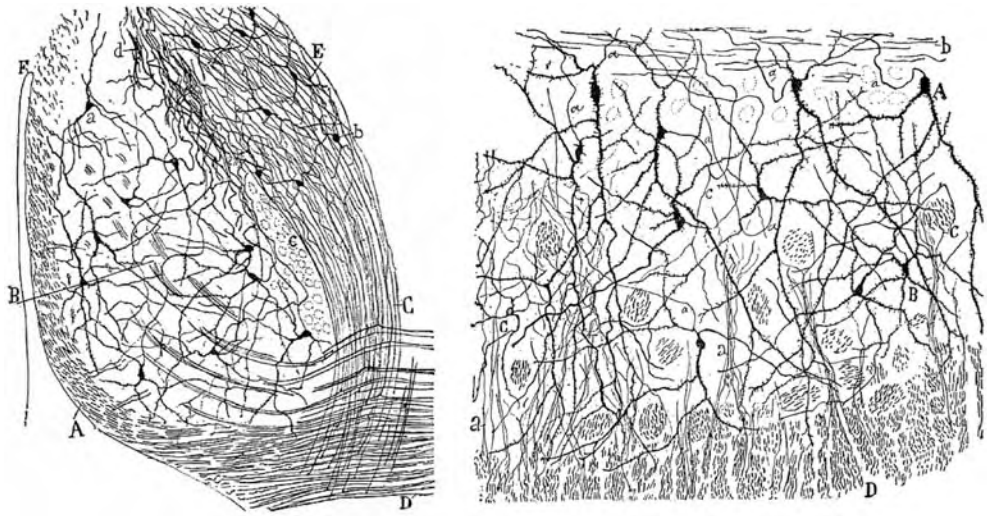

Fig. 9. Cajal's drawings of the features he observed in the ventral midbrain with Golgi impregnation. Left: Sagittal section of the mouse brain. A, cerebral peduncle; B, substantia nigra; C, bundle of collaterals destined to the infra-thalamic region; D, continuation of the cerebral peduncle; F, protuberance; d, bundle emanating from the substantia nigra. Right. Portion of a frontal section of the substantia nigra, from a kitten of a few postnatal days of age. A, upper cells; B, lower cells; C, cells with a short-axon (?) – the question mark is in the original legend; D, cerebral peduncle; a, collaterals deriving from the cerebral peduncle and ramifying in the substantia nigra. Reproduced from Cajal (1911).

cells. This kind of contact formed 'autapses' (autaptic synapses), a term introduced by van der Loos and Glaser (1972) to describe a synapse between a neuron and a collateral of its own axon.

Since the initial extensive studies based on TH immunohistochemistry (Hökfelt et al., 1976), the arrangement of dendrites extending into the SNr in bundles in which DA neurons are intertwined turned out to be a remarkable feature of dopaminergic SN neurons (Fig. 10C,D). Such an arrangement defines finger-like extensions (frequently referred to as 'columns') that penetrate deeply into the SNr.

2.2.3. Ventral tegmental area

The VTA was originally described as 'nucleus tegmenti ventralis' by Tsai (1925) in a study on the optic tract and centers of the opossum (Fig. 11). In this investigation, Tsai (1925) referred to earlier studies (Hiraiwa, 1915; Castaldi, 1923) which had regarded this nucleus 'as part of the substantia nigra'. However, Tsai (1925) described it as an independent entity, especially on the basis of its relationships with the surrounding fiber bundles, and thus stated that the 'nucleus tegmenti ventralis' differed 'from the nonspecific character of the substantia nigra connections'. Following this initial description in a marsupial, the VTA was identified in several animal species (cf. the review of Huber et al. (1943)).

According to Halliday and Törk (1986), the region of the ventromedial mesencephalic tegmentum contains approximately 27,000 cells in the rat (and approximately 47,000 cells in the monkey and 690,000 cells in the human). Swanson (1982) calculated that about 80% of these cells are TH-immunopositive, and therefore dopaminergic, in the rat VTA (see

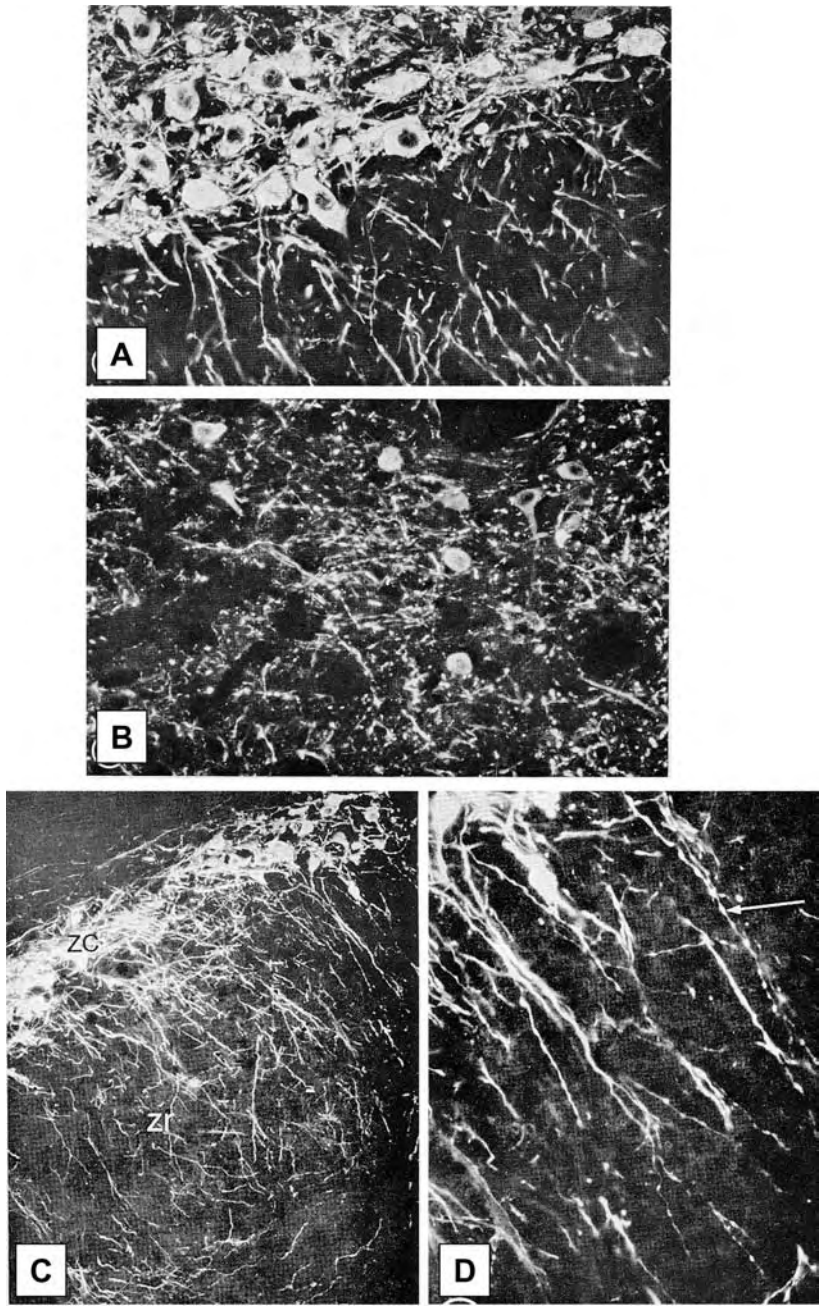

Fig. 10. The plate illustrates details of midbrain dopaminergic neurons labeled by tyrosine hydroxylase immunoreactivity. A and B illustrate a comparison between dopaminergic neurons of the substantia nigra pars compacta (A) and of the ventral tegmental area (B), showing the different sizes and packing density of these neuronal subsets. C and D show the arrangement of immunostained dendritic arborizations extending from neurons of the substantia nigra pars compacta (zc) into the pars reticulata (zr). D shows at higher magnification a detail of the upper right corner of the low power view shown in C: smooth and varicose dendrites are evident and the arrow points to one varicose process. Adpated from Hökfelt et al. (1976).

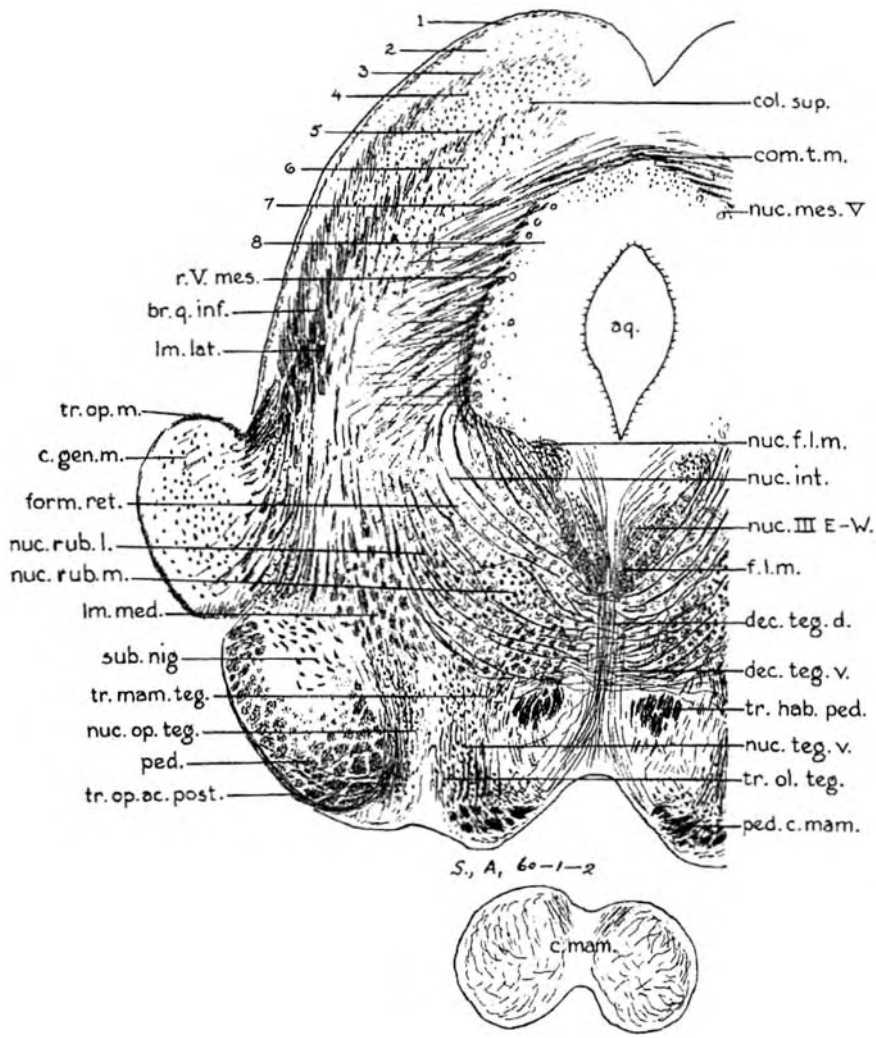

Fig. 11. The figure is reproduced from Tsai (1925) and corresponds to one of the sections through the brain of the opossum (the 'transverse section at the level just anterior to the entrance of the nervous oculomotorius' in the original legend) in which Tsai first identified and labeled the 'nucleus tegmenti ventralis', that was later denominated as 'ventral tegmental area of Tsai' and became the VTA (dropping the eponym) of the modern nomenclature. Abbreviations: aq., aqueductus cerebri; br.q.inf., brachium quadrigeminum inferius; c.gen.m., corpus geniculatum mediale; c.mam., corpus mamillare; col.sup., colliculus superior; com.t.m., commissura tecti mesencephali; dec.teg.d, decussatio tegmenti dorsalis; dec.teg.v., decussatio tegmenti ventralis; f.l.m., fasciculus longitudinalis medialis; form.ret., formatio reticularis; lm.lat., lemniscus lateralis; lm.med., lemniscus medialis; nuc.f.l.m., nucleus of fasciculus longitudinalis medialis; nuc.III E-W., nucleus nervi oculomotorii, Edinger-Westphal; nuc.int., nucleus interstitialis tegmenti; nuc.mes.V., nucleus mesencephalicus V; nuc.op.teg., nucleus opticus tegmenti; nuc.rub.l., nucleus ruber lateralis; nuc.rub.m., nucleus ruber medialis; nuc.teg.v., nucleus tegmenti ventralis; ped., pes pedunculi; ped.c.mam., pedunculus corporis mamillaris; r.V.mes., radix mesencephalica trigemini; sub.nig., substantia nigra; tr.hab.ped., tractus habenulo-peduncularis; tr.mam.teg., tractus mamillo-tegmentalis; tr.ol.teg., tractus olfacto-tegmentalis; tr.op.ac.post., tractus opticus accessorius posterior; tr.op.m, mesencephalic fibers of the tractus opticus; 1, stratum zonale; 2, stratum griseum superficiale; 3, stratum opticum; 4, stratum griseum medius; 5, stratum album medius; 6, stratum griseum profundum; 7, stratum album profundum; 8, stratum griseum centrale.

also Section 2.3). Besides the dopaminergic neurons, the VTA also contains GABAergic neurons, which project to the ventral striatum or to the prefrontal cortex (Kosaka et al., 1987; Van Bockstaele and Pickel, 1995; Carr and Sesack, 2000a) (see Sections 7.2 and 8.2).

In the ventromedial mesencephalic tegmentum, cells are rather loosely arranged (Figs. 4–6), and Halliday and Törk (1986) evaluated that the packing density of the SNc is about twice than in the VTA. In this latter area the cells are small-sized, ranging from 6 to 26 μm in the rat (from 4 to 34 μm in the monkey, and from 10 to 53 μm in the human), exhibiting in Nissl-stained sections a variety of staining intensities and shapes (round, ovoid, fusiform, stellate, polygonal or irregular) (Halliday and Törk, 1986).

In the Nissl-stained sections, the VTA appears continuous with the dorsal portion of the SNc (Phillipson, 1979a; Figs. 4–6). With Golgi impregnation (Phillipson, 1979b), some heterogeneity was found in the cells of the different VTA components (represented by the nuclear subdivisions listed in Section 2.1.1), with a main dendritic organization approximately in the horizontal plane. Although the VTA merges laterally with the SNc, Phillipson (1979b) emphasized that in the VTA, there is no clear counterpart to the SNr and neurons do not have long, ventrally directed dendrites.

2.3. A8, A9 AND A10 CELL GROUPS

On the basis of their observations with histofluorescence, Dahlström and Fuxe adopted in 1964 a new nomenclature for the monoamine-containing cell groups. For descriptive purposes, the catecholamine class of monoamines were given the 'A' (dopamine and noradrenaline) or 'C' (adrenaline) designation, and the indoleamine class of monoamines were defined as 'B' (serotonin) cell groups. The monoamine-containing cell groups were also numbered sequentially according to their caudorostral distributions from the medulla oblongata to the diencephalon. This new nomenclature was due to the fact that the distribution of neurons exhibiting fluorescent labeling appeared to cross anatomical boundaries, so that a precise correspondence with anatomically identified structures was difficult to determine. In addition, cytoarchitectonic features of the unlabeled structures surrounding monoaminergic cell groups were probably difficult to define under fluorescence observation.

The DA-containing system of the midbrain was divided in the rat by Dahlström and Fuxe (1964) in the A8, A9 and A10 cell groups (Fig. 2). As mentioned above, this nomenclature is still widely in use. The A8 cells are predominantly found in the RRA, whereas the subdivision into A9 and A10 cell groups was based on a lateral-medial topography. The A9 neurons are located in the SNc with some neurons extending in the SNr and SNl (Fig. 7). The A10 cells are located in the VTA, extending into the structures located at the midline or closer to it, mentioned in Section 2.2.1 (Figs. 7, 8) (see also Hökfelt et al., 1984a). In both the rat (Fig. 10A,B) and the mouse (Nelson et al., 1996) the cells identified as dopaminergic are smaller in the A10 cell group than in the A9 cell group.

Dopaminergic neurons of the A8 cell group, orginally defined by Dahlström and Fuxe (1964) as supralemniscal cells, are located dorsal and caudal to the SN (Fig. 2). The A8 neurons are generally considered to represent an extension of the A9 cell group, since the rostral and ventral portion of the A8 cell group cannot be clearly differentiated from the contiguous A9 cells of the caudal and lateral SN. The A8 cells are also continuous with the caudal and lateral portions of the A10 cell group extending in the parabrachial pigmented nucleus. Retrorubral neurons, visualized by intracellular filling in the cat,

appeared as medium sized and sparsely branched neurons, with long dendrites and distally located spine-line appendages, similar to the SNc neurons (Preston et al., 1981).

In the rat, the A8 neurons form rostrally a cell bridge, which joins the A9 and A10 cells and includes the neurons embedded in the fascicles of the medial lemniscus. The A8 neurons are organized caudally in a ventral cell sparse region which then disappears, and a dorsal cell dense portion which extends further caudally to the mesopontine junction (Deutsch et al., 1988). The position of the A8 cell group remains roughly comparable across the mammalian species (Deutsch et al., 1988), including the mouse (Nelson et al., 1996) (Fig. 8). Dopaminergic neurons of the A8 cell group contribute efferents to all the forebrain dopaminergic pathways (Deutsch et al., 1988): they give origin to projections to the striatum as part of the nigrostriatal cell population (see Section 5.2), as well as to the mesolimbic pathways (see Section 7.2) and to projections to the cerebral cortex (see Section 8.2).

Björklund and Lindvall (1984) reported that in the rat TH immunohistochemistry reveals 15,000–20,000 dopaminergic neurons on each side of the midbrain tegmentum, and about 9000 of these cells belong to the VTA. Despite the unavoidable variability of cell counts, subsequent investigations in the rodents are in keeping with these quantitative figures. German and Manaye (1993) evaluated a total number of approximately 45,000 TH-immunoreactive neurons bilaterally in the rat midbrain. In the mouse, marked differences in the number of midbrain dopaminergic (TH-immunoreactive) cells have been reported in different strains (Záborsky and Vadasz, 2001). For example, the total number of these cells varies from approximately 21,000 in C57BL/6 mice to 30,000 in FVB/N mice, with no differences in the volume of the striatum between these two strains (Nelson et al., 1996).

In terms of relative proportion of TH-immunopositive cells, the A8 cells account for about 5%, and the A9 and A10 cells account for about 95%, with a more or less equal distribution in rodents. In particular, the A10 cells account for 46% of the total number of midbrain dopaminergic neurons in the rat (German and Manaye, 1993), and 50–52% in the mouse (Nelson et al., 1996). These studies (German and Manaye, 1993; Nelson et al., 1996) also emphasized that the proportion of DA-containing cells located in nuclei A8, A9 and A10 differs greatly from rodents to primates. In the primates (with a total number of 160,000 TH-immunoreactive neurons in the macaque monkey midbrain, and estimates ranging from 400,000 to approximately 600,000 in the human midbrain), the majority ($>70\%$) of midbrain dopaminergic neurons are located in the A9 cell group. In the primates, therefore, the A9 region seems to undergo a considerable expansion compared to the rodents.

2.4. THE DORSAL AND VENTRAL TIERS

The distinction of the midbrain dopaminergic system into a dorsal and a ventral tier is based on the main cellular features mentioned above, as well as on distinct neurochemical features and pattern of connectivity, which will be presented in the following sections but are summarized here.

Tiers or 'sheets' of dopaminergic cell bodies were initially defined in the rat mainly on the basis of their projections. By the use of anterograde and retrograde tracers, Fallon and Moore (1978) observed that the A9 and A10 neurons formed a continuum, with both cell groups contributing to the nigrostriatal, mesolimbic and mesocortical pathways (Fig. 12), and were arranged in a dorsal to ventral gradient in the neural origin and termination

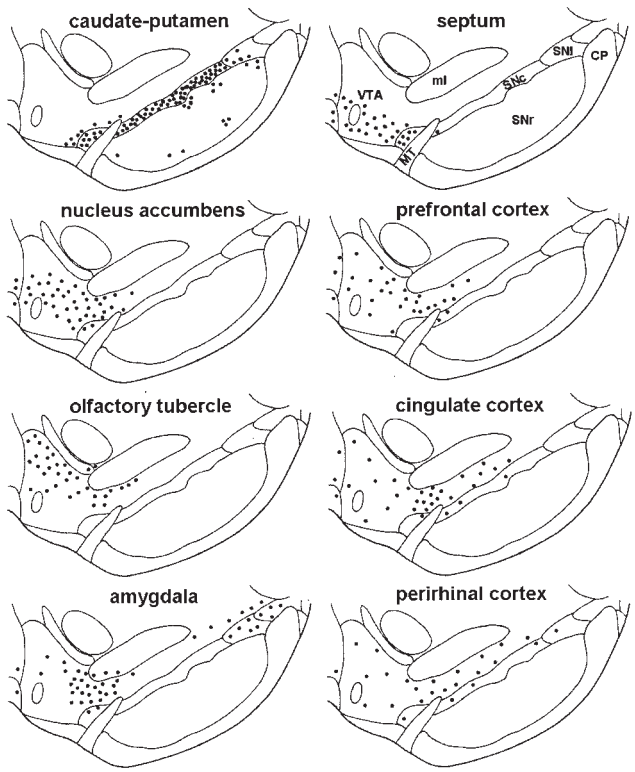

Fig. 12. The diagram, redrawn from Fallon and Loughlin (1995), summarizes the distribution in the rat of midbrain dopaminergic neurons which give origin to different sets of telencephalic projections. Abbreviations: CP, cerebral peduncle; ml, medial lemniscus; MT, medial terminal nucleus of the accessory optic tract: SNc, substantia nigra, pars compacta; SNl, substantia nigra, pars lateralis; SNr, substantia nigra, pars reticulata; VTA, ventral tegmental area.

field. Therefore, the origin of efferents of midbrain dopaminergic cells encompassed not only cytoarchitectonic boundaries but also the subdivisions originally made with histofluorescence.

In the rat, the dorsal tier includes cells of the dorsal parts of the VTA and SNc and cells of the RRA innervating the limbic portion of the striatum and limbic cortical fields, as well as the ventral basal forebrain structures, such as the olfactory tubercle and the amygdala. Neurons of the dorsal tier are mostly fusiform, with dendrites oriented horizontally in the mediolateral plane of the SNc. From the neurochemical point of view, neurons of the dorsal tier contain relatively low levels of TH mRNA and dopamine transporter (DAT) mRNA, and the calcium binding protein calbindin is colocalized with DA in most dorsal tier neurons (Gerfen, 1985) (Figs. 4D; 13C,D).

The ventral tier includes in the rat cells of the ventral parts of the VTA and SNc which innervate the neostriatum and dorsal structures of the basal forebrain such as the septum. Ventral tier neurons include the 'columns' of dopaminergic neurons which pierce the SNr and project to the striatum (Figs. 12, 13A). The ventral tier neurons express high levels of DAT mRNA and do not exhibit calbindin immunoreactivity (Gerfen, 1985).

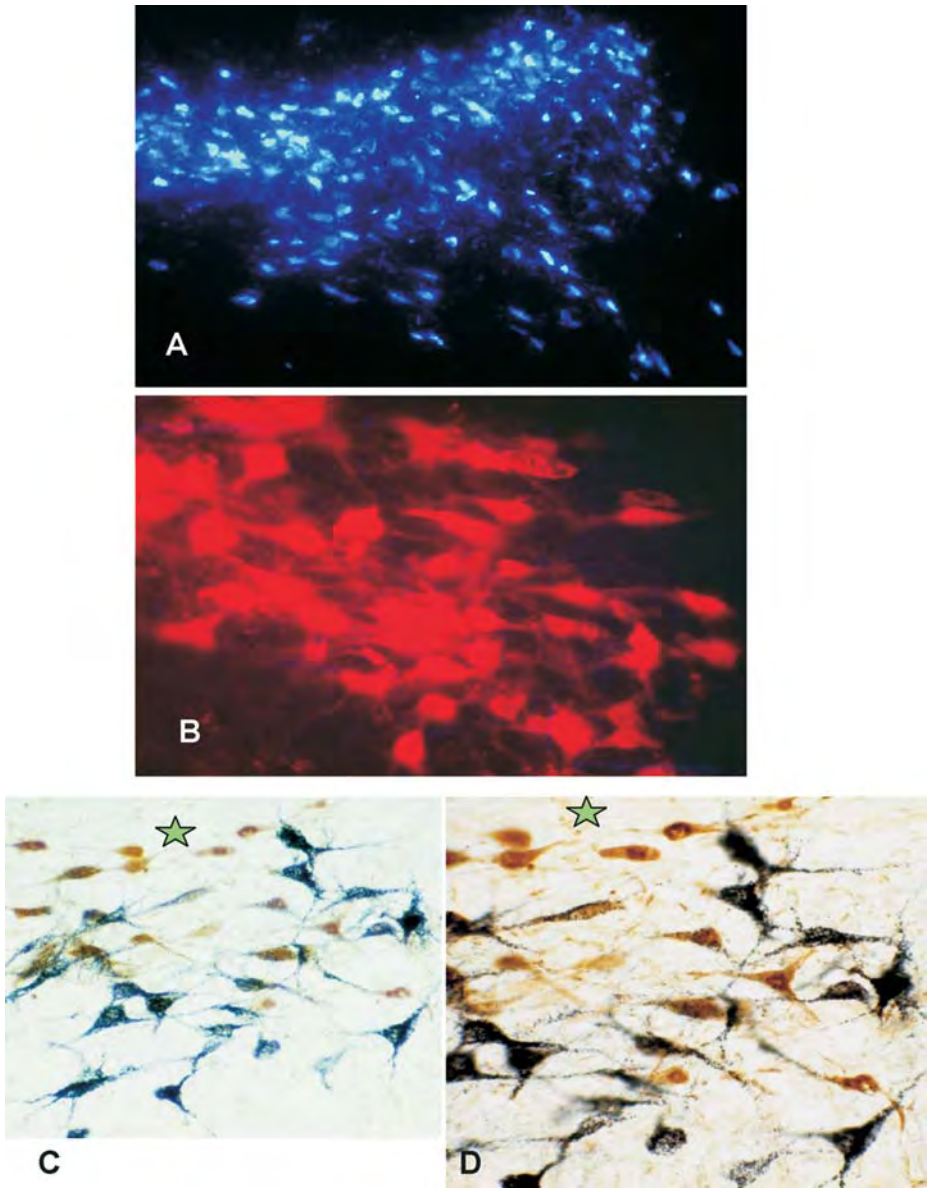

Fig. 13. The plate illustrates the features of neurons of the substantia nigra pars compacta (SNc) projecting to the striatum, as revealed by retrograde labeling after large injections of tracers in the rat striatum. A is a low power view of the SNc labeled by the fluorescent tracer Fast Blue: note the so-called 'columns' or 'fingers' of SNc cells extending into the (unlabeled) pars reticulata of the substantia nigra. B is a higher power view of SNc nigrostriatal neurons labeled by the fluorescent tracer Evans Blue: note the different cell shapes and sizes. C and D are microphotographs of SNc neurons in a double labeling experiment in which calbindin immunoreactivity (visualized by the brown reaction products of the chromogen 3′-3′ diaminobenzidine) was combined with retrograde labeling with the tracer wheat germ agglutinin conjugated with enzymatically inactivated horseradish peroxidase and with colloidal gold (revealed by the black granules resulting from silver enhancement). D is a higher magnification of the upper part of C, and the star labels the same point for spatial reference. Note that there are single labeled neurons of each cell population (calbindin-immunoreactive or retrogradely labeled) and double labeled neurons containing black granules in a brown cytoplasm. Note in C the dorsal location of calbindin-immunostained neurons (which correspond to the dorsal tier of midbrain dopaminergic neurons).

At rostral levels (Fig. 4), the dorsal and the ventral tiers of the SNc are both located dorsal to the SNr, where they are distributed in two sheets of neurons one on top of the other. Proceeding caudally, the ventral tier of the SNc splits into two parts, one subjacent to the cells of the dorsal tier and the other comprising the dopaminergic neurons located within the SNr. These caudal dopaminergic neurons are also well evident in the mouse (Fig. 8).

The spatial arrangement of the dopaminergic cells of the dorsal and ventral tiers and their projections will be dealt with again in relation to the nigrostriatal cell population (see Section 5.2). It is, however, important to mention here that the features of connectivity with the striatum strengthen the subdivision into dorsal and ventral tiers, whose neurons give origin to axonal subsets differentially organized in terms of their termination in the striatal compartments (see Section 5.2).

2.5. SYNAPTIC FEATURES: DENDRITIC RELEASE OF DOPAMINE AND ELECTRICAL SYNAPSES

2.5.1. Dendrodendritic synaptic contacts

It is now well ascertained that dendrites are capable of propagating action potentials not only in distal to proximal direction, but also in the reverse direction by back-propagation after initiation at the cell body (Ludwig and Pittman, 2003). The so-called 'law of dynamic polarization' enunciated by Cajal (see Berlucchi, 1999) was aimed at stating the unidirectional propagation of excitations within the nervous system, and assumed that nerve impulses are conducted from the dendrite or soma to axon terminals. This dogma is now being reconsidered, not only in view of the evidence of dendrodendritic synapses, but also in view of the existence of electrical synapses in which the flow of information can be bidirectional.

Since the description in the vertebrate olfactory bulb (Rall et al., 1966), the occurrence of presynaptic dendrites has been reported in a variety of central nervous system (CNS) regions. The SN was one of the structures in which dendrodendritic contacts were first observed (Björklund and Lindvall, 1975), and demonstrated at the electron microscopic level (Hajdu et al, 1973; Wilson et al., 1977; Groves and Linder, 1983). DA was one the first neuroactive substances shown to be released from dendrites (Groves et al., 1975; Geffen et al., 1976), and, as reviewed by Cheramy et al., (1981), local dendritic release of DA in the SN was firmly established since the initial studies on this neurotransmitter.

Groves and Linder (1983) made a number of interesting ultrastructural observations based on the labeling of dopaminergic dendrites with the false neurotransmitter 5-hydroxydopamine (which is taken up by monoaminergic neurons and forms an electron-dense core within synaptic vesicles and other membrane-bound cell compartments). Exploiting this strategy, Groves and Linder (1983) described that dendrodendritic synapses represented a small proportion of the total synapses in the SN, and that, at variance with the dendrodendritic contacts in other brain regions, in the SN these contacts did not appear to engage in reciprocal or serial synapses.

For many years dendritic release of the neurotransmitter was considered a peculiarity of the midbrain dopaminergic neurons. However, this mechanism of release has now been ascertained for other neuroactive substances as well, including other neurotransmitters.

The exocytotic machinery of dendritic release, which differs from that of axon terminals, is at present the subject of extensive investigation (Ludwig and Pittman, 2003).

Local modulation through the dendritic release of DA occurs in both the SN and the VTA. Ultrastructural observations have been made with immunolocalization of the vesicular monoamine transporter-2 as marker for sites of intracellular monoamine storage within SN and VTA dopaminergic neurons identified by TH immunoreactivity (Nirenberg et al., 1996a). This study has reported that DA is stored in and may be released from dendritic small synaptic vesicles or large dense-core vesicles, while the smooth endoplasmic reticulum represents the main site for the DA storage.

An inhibitory postsynaptic current elicited by somatodendritic DA release has been recently reported using whole-cell recordings from dopaminergic cells in slices of the ventral midbrain from mouse (Beckstead et al., 2004). The data obtained in this study indicated that depolarization of the dopaminergic cells activates the calcium influx through voltage-sensitive channels, releasing DA from somatodendritic vesicular stores to act on DA autoreceptors. In addition, the study of Beckstead et al. (2004) indicates that synaptic DA transmission directly regulates cell excitability, that is mediated through exocytosis, and that does not depend on volume transmission and acts instead in a localized area.

2.5.2. Connexin 36 expression in midbrain dopaminergic cells and gap junctions

Gap junctions are the sites of intercellular membrane channels which provide for direct cytoplasmic continuity between the adjacent cells (Simon and Goodenough, 1998). A wealth of recent data have indicated that connexins are the proteins assembled into gap junction channels, and represent the building blocks of these channels (see the reviews of Bennett, 1997; Hormuzdi et al., 2004).

Gap junctions provide in the nervous system the structural correlate of one class of electrical synapses, characterized by very close apposition between the presynaptic and postsynaptic membranes. It should be noted, in this respect, that different junctional specializations can mediate different forms of electrical transmission between neurons (Bennett, 1997). Electrical synapses transmit preferentially, but not exclusively, low-frequency stimuli, that allow the rapid transfer of a presynaptic impulse into an electrical excitatory potential in the postjunctional cells. Electrical transmission, via the intercellular channels, can be bidirectional. The widely held opinion that electrical transmission is characteristic of lower vertebrates probably derives from the large cell systems in which electrical synapses were identified in the initial period of intracellular recording (reviewed by Bennett, 1997). Contradicting this view, electrotonic coupling between neurons has now been demonstrated in many areas of the mammalian central nervous system and has been implicated in neuronal synchronization. Gap junctional intercellular communication can occur between glial cells, glia and neurons, as well as between neurons.

Connexins are tetra-pass membrane proteins that oligomerize into hexameric hemichannels called connnexons. These gap junction proteins are encoded by a multigene family. The presence of gap junctions and the expression of connexins has been described in many areas of the developing and adult central nervous system. Up to now, only connexin (Cx) 36 and Cx45 have been found to be expressed in neurons, besides Cx43 which is expressed in the olfactory epithelium.

The distribution of Cx36 mRNA has been mapped in the rat and human nervous system with in situ hybridization (Condorelli et al., 2000). Cx36 expression was found to

be very high in the inferior olive, and was also detected in several other areas. High expression of Cx36 mRNA was detected in the SNc and in the VTA, as well as in the SNr. Double labeling of Cx36 mRNA and TH immunoreactivity confirmed that this gap junctional protein was expressed by dopaminergic neurons (Fig. 14).

It is also worth noting that in other key structures of basal ganglia circuits, such as the caudate-putamen, nucleus accumbens (NAc) and globus pallidus (GP), Cx36 expression was found in subpopulations of scattered cells (Condorelli et al., 2000). These findings are in agreement with the report of dye and electrotonic coupling in the NAc (O'Donnell and Grace, 1993). However, altogether, these data indicate that the midbrain dopaminergic neurons represent the main center in which electrical synapses are utilized for intercellular communication within the basal ganglia.

The dye and electrotonic coupling between pairs of dopaminergic neurons has been reported in the SNc, and it has been suggested that electrical communication between these neurons could be involved in burst firing and in the synchronization of the DA release (Grace and Bunney, 1983; Freeman et al., 1985; Freeman and Bunney, 1987). In a recent study based on chronic electrical recording in the freely moving rats (Hyland et al., 2002), simultaneous activation of midbrain dopaminergic neurons was found to be a rare phenomenon. However, the data obtained in slices have pointed out that in dopaminergic neurons of the rat midbrain, coactivation of glutamate receptor subtypes can transform a temporally dispersed GABAergic input into a rhythmic pattern of firing, probably through a mechanism involving electrotonic couplings (Berretta et al., 2001). The availability of Cx36 as a novel tool for the identification of neurons which build up gap junctional proteins, can now open new perspectives in the investigation of this mechanism of communication between dopaminergic cells in health and disease.

2.6. GLIAL CELLS INHABITING DOPAMINERGIC CELL GROUPS IN THE MIDBRAIN

Since DA is contained in and synthesized by neurons, the features of the glial cells which surround dopaminergic neurons are in general neglected when dealing with these cells. Interest is instead focused on both the neurons and glial cells in the studies dealing with the neurotoxic, neuroinflammatory and neurodegenerative alterations, which affect midbrain dopaminergic cells. It should, however, be emphasized that glial cells, both astrocytes and microglia, represent major components of cell groups of the ventral midbrain tegmentum, as elsewhere in the normal brain. The crosstalk between glia and neurons, including a key role of glia in neurotransmission, is now receiving increasing attention (see, for example, Haydon, 2000).

In relation to the glia which co-inhabit the mesencephalic tegmentum in the normal brain, it is interesting to note that studies on astrocytes and microglia have pointed out peculiarities of the latter type of glial cells. Astrocytes, investigated in the rat brain, did not exhibit high density in the mesencephalon and in particular in the SN (Savchenko et al., 2000). Studies in both the mouse (Lawson et al., 1990) and the rat (Kim et al., 2000) reported instead that microglia has a very high density in the SN. In the mouse, the microglial cells were also found to be very dense in the other basal ganglia structures, such as the striatum (Lawson et al., 1990).

In the recent years microglial cells have received a wealth of attention in relation to their role as resident immune cells in the brain (Raivich et al., 1999; Streit, 2002). They are protagonists of the immune surveillance in the CNS, and virtually any inflammatory,

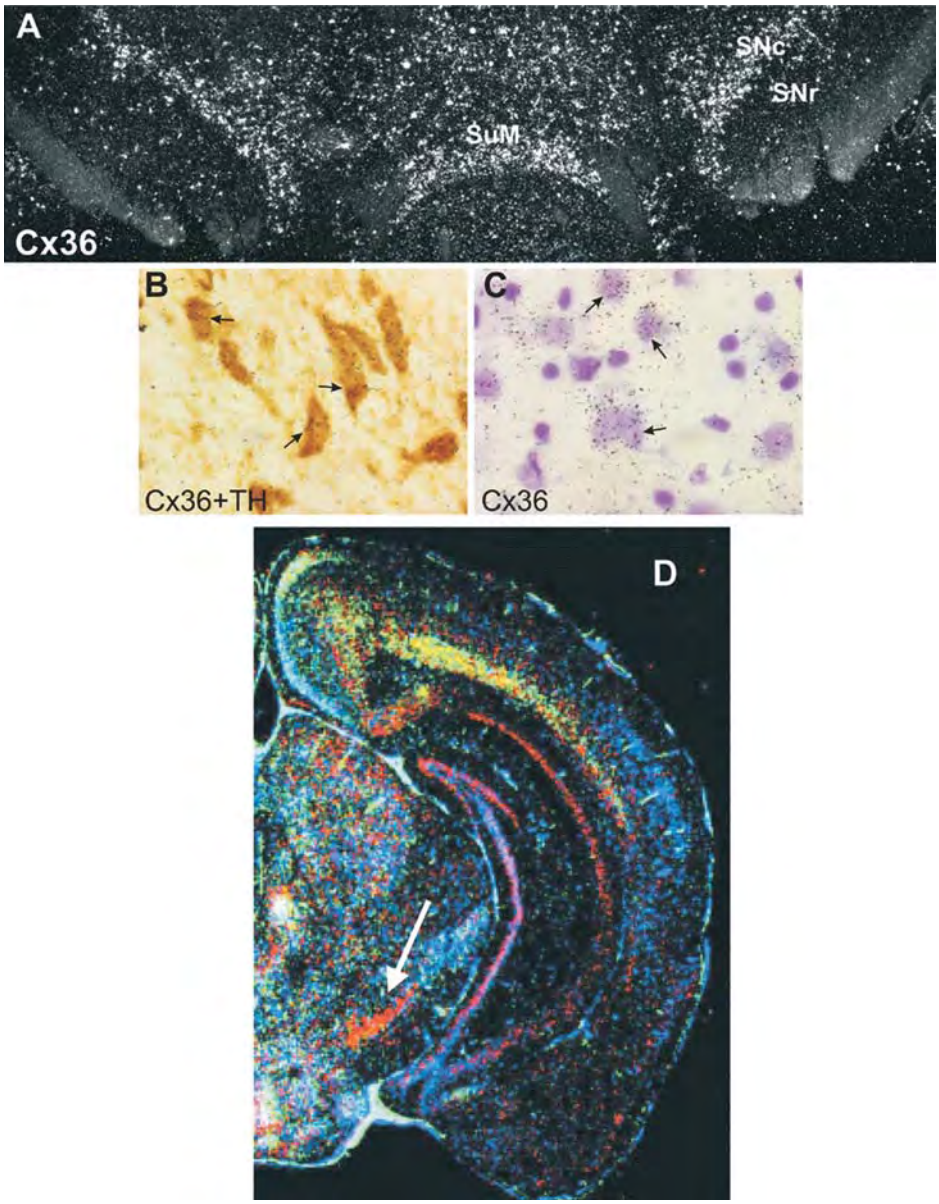

Fig. 14. The plate illustrates the signal obtained with in situ hybridization for Cx36 mRNA in the adult rat mesencephalon (A–C), and the expression of mRNAs of major histocompatibility complex (MHC) class I molecules in the adult mouse brain (D). A: Note in the dark-field microautoradiograph the intense labeling of the substantia nigra pars compacta (SNC), in contrast to the low signal in the pars reticulata (SNr); high signal is also seen in the supramammillary nucleus (SuM). B: The image shows that Cx36 mRNA (black grains) is expressed in dopaminergic neurons of the SNc, identified by tyrosine hydroxylase (TH) immunoreactivity (brown staining); the arrows point to some of the double labeled cells. C: Bright-field image of SNc neurons (cresyl violet staining) labeled by Cx36 mRNA signal (black grains); the arrows point to labeled neurons with relatively large nuclei. This figure was kindly provided by N. Belluardo, G. Mudò, and D.F. Condorelli. D: The coronal section illustrates the distribution of mRNAs for different MHC class I molecules (blue: H-2D; green: Qa-1; red: T22). Note the high expression of MHC class I T22 mRNA in the pars compacta of the substantia nigra (arrow). Adapted with permission from Boulanger and Shatz (2004).

infectious or toxic stimulus affecting a neuron of the CNS triggers microglia activation. Immediately after a challenge, the microglia release neurotrophic factors that promote recovery of the injured neurons. When the noxious stimulus elicits an irreversible damage, the neuronal signals induce microglia to produce toxic factors, thus accelerating neuronal degeneration and removal of debris by phagocytosis.

Inflammatory responses mediated by microglia, which also trigger oxidative phenomena, are raising growing interest in relation to their role in neurodegenerative disorders, including Parkinson's disease (see, for example, Gonzalez-Scarano and Baltuch, 1999). The abundance of microglia in the SN of the normal brain may, therefore, represent an important feature for the vulnerability of midbrain dopaminergic neurons to different kinds of insult. For example, the high density of microglia in basal conditions has been related to the susceptibility, higher in the midbrain DA-containing cell groups than in other brain sites, to a proinflammatory challenge, such as that provoked by injection of the bacterial endotoxin lipopolysaccharide (Kim et al., 2000). A regional vulnerability to oxidative damage, with a key role of microglia, has also been implicated in the susceptibility of midbrain dopaminergic neurons to the exposure to environmental agents, which is raising increasing interest as potential pathogenetic factor of Parkinson's disease (Gorell et al., 1998; Di Monte et al., 2002). It is interesting to recall in this context that the chronic administration of rotenone, a common herbicide, results in selective destruction of the nigrostriatal dopaminergic cells in the rat (Betarbet et al., 2000), and microglial cells intermingled with dopaminergic neurons have been demonstrated to play a pivotal role in the selective neurodegenerative ability of this pesticide (Gao et al., 2002).

3. NEUROCHEMICAL FEATURES OF THE MIDBRAIN DOPAMINERGIC CELL GROUPS AND THEIR INPUTS

3.1. A TERRITORY WITH A RICH MOLECULAR REPERTOIRE AND TARGETED BY DIVERSE AFFERENT INPUTS

A complete account of the spectrum of neuroactive molecules expressed in midbrain dopaminergic neurons and contained in the fibers which innervate the ventral midbrain tegmentum would require a chapter in itself and goes beyond the scope of the present overview. These molecules include classical neurotransmitters, such as γ-amino-butyric acid (GABA) and glutamate, as well as numerous neuromodulators. The activity of midbrain dopaminergic neurons is governed by a balance between excitatory and inhibitory inputs, and a significant proportion of these inputs is mediated through GABA and glutamate receptors. DA receptors also play an obvious key role in the SN and VTA, and the different classes of these receptors expressed in the ventral midbrain tegmentum will be dealt with in the last part of this chapter.

We wish to briefly recall here that the dopaminergic midbrain cell groups, as well as the SNr, are recipients of inputs within basal ganglia circuits (see Section 4), and from other sources (see for review Fallon and Loughlin, 1995). In particular, the main GABAergic input to the SN is derived from the medium-sized spiny neurons of the striatum. The striatonigral pathway terminates densely upon GABAergic neurons of the SNr, and more sparsely in the VTA, SNc and SNl. As it will be dealt with in Section 5.1, striatonigral neurons projecting to the SNr and SNc reside in different compartments of the striatum. Neuropeptides are colocalized with GABA in the striatal cell bodies and fibers which

innervate the SN (see Section 3.4). Fibers deriving from the globus pallidus (GP) and ventral pallidum give rise to a less dense GABAergic projection to both the SNc and the SNr. The main excitatory input to the SN arises from the subthalamic nucleus (STh), and these afferents have a distribution similar to the GABAergic striatal input, being very dense in the SNr, and sparsely distributed to the SNc.

Projections to the SNc and VTA also originate in the amygdala, hypothalamus, frontal and cingulate cortical areas, providing inputs which could play a critical role in the integration of cognitive, emotional, autonomic and motor components of behavior. In particular, the amygdalonigral pathway originates in rat in a discrete region of the central nucleus of the amygdala which extends rostrally into the so-called 'extended amygdala' (see Section 7.3). Amygdaloid fibers terminate in the SNc and in the SNl, but not in the SNr (Gonzalez and Chesselet, 1990). The SNc is also innervated by fibers originating in the lateral habenula (Herkenham and Nauta, 1979).

Recent ultrastructural findings on the synaptic organization of the projections from the prefrontal cortex to the VTA in the rat have revealed selective targeting of specific neuronal populations in the VTA (Carr and Sesack, 2000b). In this study, prefrontal fiber terminals were seen to establish asymmetric synapses on dopaminergic and GABAergic VTA neurons whose target sites were not identified; however, a subset of prefrontal terminals established synaptic contacts with GABAergic VTA neurons that project to the nucleus accumbens (NAc) (see Section 7.2), and another population of prefrontal terminals on dopaminergic VTA neurons that project back to the prefrontal cortex (see Section 8.2).

It has been recently reported in the rat that the SNc receives projections from the superior colliculus (Comoli et al., 2003). In this study, tectonigral fibers were seen to establish both asymmetric and symmetric synaptic contacts on TH-positive and TH-negative elements in the SNc. The anatomical and electrophysiological findings of this investigation indicated that visual information relayed to the DA-containing midbrain neurons could be involved in the critical perceptual discriminations that identify biologically salient events.

Cholinergic inputs to dopaminergic cells derive mainly from the nuclei located at the mesopontine junction, namely the pedunculopontine and laterodorsal tegmental nuclei (review in Fallon and Loughlin, 1995), and it has been shown in primates that these nuclei provide also a glutamatergic input to the SN (Lavoie and Parent, 1994). Cholinergic fibers innervate rather densely the SNc and SNl, and are also distributed in the VTA but are very sparse in the SNr. Nicotinic acetylcholine receptors are very dense throughout the VTA, SNc and SNl (Fallon and Loughlin, 1995; Golner et al., 1997). Nicotinic cholinergic modulation of dopaminergic transmission is considered to underlie the addictive properties of nicotine, the drug of abuse contained in cigarette smoke. Muscarinic receptors are sparse and, in particular, dopaminergic SN and VTA neurons express M5 receptors (Yeomans et al., 2001).

Studies based on dual immunolabeling in electron microscopy have confirmed that cholinergic axons terminate on dopaminergic neurons in both the SNc (Bolam et al., 1991) and the VTA (Garzón et al., 1999), establishing asymmetrical synaptic specializations with dendrites and perikarya of dopaminergic cells. Exploiting dopamine transporter (DAT) immunoreactivity to identify dopaminergic VTA neurons and vesicular acetylcholine transporter immunoreactivity to identify cholinergic fibers, Garzón et al. (1999) reported targeting of cholinergic afferents to both nondopaminergic and dopaminergic VTA neurons, and in particular to a subpopulation of dopaminergic neurons expressing low levels of DAT. Since the latter feature has also been reported in dopaminergic axons

innervating the rat frontal cortex (Sesack et al., 1998), these data suggest that cholinergic fibers target the VTA neurons of origin of cortical innervation (see Section 8.2).

Dopaminergic cell groups of the ventral midbrain tegmentum are also innervated by other monoamine-containing fibers. As initially reported by Phillipson (1979c), the serotonin innervation of the SN and the VTA arises from the dorsal and median raphe nuclei (see also Halliday and Törk, 1989). Serotonin receptors are distributed throughout the SNr. In addition, immunoreactivity to the serotonin receptor subunit 5-HT_{2A} has been described in the rat throughout the dopaminergic A10 cell population, which could be relevant for DA and serotonin interactions potentially implicated in psychiatric disorders and drug abuse (Nocjar et al., 2002).

Noradrenergic fibers efferent from the locus coeruleus are very scarce in the midbrain dopaminergic cell groups, where they provide a sparse innervation of the VTA; moderate levels of α- and β-adrenoceptor binding sites are present in the SN (reviewed by Marien et al., 2004). These findings are in contrast with the wealth of evidence indicating that the noradrenergic system influences the activity of the nigrostriatal dopaminergic system (as also shown by the electrophysiological findings of Paladini and Williams, 2004). Therefore, alternative multisynaptic pathways (with relays in the raphe nuclei or in the striatum) have been proposed for noradrenaline–DA interaction (Marien et al., 2004).

Opioid receptors (and especially μ receptor binding) are abundant in all the subregions of the ventral midbrain tegmentum, including the SNr (Fallon and Loughlin, 1995).

Several data indicate that the activity of midbrain dopaminergic neurons is highly regulated via interactions of neurotransmitters with their receptors. For example, although activation of muscarinic receptors is known to activate dopaminergic neurons enhancing DA release, presynaptically located muscarinic receptors can modulate excitatory transmission to neurons of the SNc and VTA (Grillner et al., 1999). In addition, the activation of muscarinic receptors and metabotropic glutamate receptors on the midbrain dopaminergic cells can result in both inhibition and excitation, depending on the extent of calcium buffering and the duration of agonist application (Fiorillo and Williams, 2000). Also glutamate can mediate inhibition or excitation in midbrain dopaminergic neurons by activation of the same receptor, depending on the frequency and pattern of input (Fiorillo and Williams, 1998). Therefore, glutamate is not exclusively an excitatory neurotransmitter but can have a dual function in synaptic transmission. Recent data obtained in slices indicate that also the noradrenergic innervation of dopaminergic cells can inhibit directly their activity (Paladini and Williams, 2004).

We will deal in greater detail with a few additional neuroactive molecules, selected on the basis of their interest in the chemical signature of dopaminergic circuits, or in view of the potential implication of midbrain dopaminergic cells in physiological regulation as well as in disease.

3.2. DOPAMINE TRANSPORTER

Besides the immunohistochemical revelation of dopaminergic cells with TH (see Sections 1.1 and 2), anti-DA antibodies were also introduced (Geffard et al., 1984). These antibodies stain preferentially the DA-containing neurons, although DA is present as a precursor also in the other catecholaminergic cells. In addition, anti-DA antibodies have some fixation requirements (DA would diffuse out of the cell unless glutaraldehyde is used as fixative), which renders difficult the combination of DA immunohistochemistry with the immunohistochemical revelation of other antigens or other labeling techniques.

Growing interest was and still is raised by neurotransmitter transporters, both for the visualization of neurotransmitter-containing cell populations and for the implications of these molecules in synaptic communication.

The mechanisms of rapid removal of neurotransmitters from the synaptic cleft terminate neurotransmission, and represent, therefore, a critical component of neuronal signaling. The sodium-dependent DAT, which removes DA from the extracellular space by active high affinity sodium-dependent reuptake, is largely responsible for the termination of DA neurotransmission. This mechanism leads to reaccumulation of DA into the presynaptic terminal, thus playing a key role in DA recycling (Uhl et al., 2003). Several psychoterapeutic drugs, and drugs of abuse, such as cocaine and amphetamine bind to DAT with high affinity. In addition, DAT transports the MPTP toxin into the dopaminergic neurons, and thus plays a role in determining the vulnerability of these neurons to MPTP toxicity, as indicated by the finding that DAT mRNA is low in midbrain regions spared by MPTP-induced degeneration in the mouse (Sanghera et al., 1994).

In both rodents and primates, DAT expression provides a specific marker of dopaminergic elements. In fact, DAT mRNA was found to be expressed only in neurons which utilize DA as neurotransmitter (Augood et al., 1993; Lorang et al., 1994; Ciliax et al., 1995; Freed et al., 1995), and DAT immunoreactivity was not detected in noradrenergic cell bodies (Ciliax et al., 1995).

In particular, in the rat DAT mRNA was found to be very intensely expressed by the A8, A9 and A10 cell groups (Lorang et al., 1994). DAT immunoreactivity based on the use of specific antibodies resulted in labeling of mesencephalic dopaminergic cells, with the exception of the medial VTA, as well as of their axons and terminal fields, although less intense than TH immunoreactivity (Ciliax et al., 1995; Freed et al., 1995). Interestingly, many DAT-immunostained dendrites were seen to descend from the SNc into the SNr, supporting a DA uptake mechanism on SNc dendrites in this region (see Section 2.5.1). In contrast, little or no DAT mRNA (Lorang et al., 1994) or immunoreactivity to DAT protein (Ciliax et al., 1995) was detected in the hypothalamus. The latter finding provided further indication that the hypothalamic dopaminergic system is independent from the midbrain dopaminergic system.

At the ultrastructural level, DAT, investigated with the immunogold technique (Nirenberg et al., 1996b, 1997a), was found to be mainly localized within perikarya and proximal dendrites of dopaminergic neurons (double labeled by immunoperoxidase reaction product for TH). In both the VTA and the SN, DAT was found to be associated with intracellular membranes of organelles (relatively large vesicles and tubulovesicles) distant from the plasma membrane, suggesting a regulation of intracellular DA storage pools. Localization on the plasma membrane was instead detected in more distal dendrites, presumably playing a role in the regulation of extracellular DA concentration. Plasmalemmal DAT immunolabeling was found in dendrodendritic appositions much more commonly in the VTA than in the SN, which is of special interest since, as mentioned above, dendritic appositions are potential sites for DA release.

3.3. CALCIUM BINDING PROTEINS

The calcium binding proteins calbindin D28k, calretinin and parvalbumin are members of a family of proteins characterized by the presence of calcium binding EF-hand motifs, modulated by stimulus-induced increases in cytosolic free calcium ions (Persechini et al.,

1989). These proteins are expressed by cell populations of the CNS (see, for the rat brain, Celio, 1990; Resibois and Rogers, 1992), and they have become widely used markers of neuronal subsets.

Parvalbumin, which is frequently colocalized with GABA in subpopulations of inhibitory neurons, in the rat SN is expressed in cell bodies of the SNr, which are GABAergic (Gerfen et al., 1985). Calbindin and calretinin are instead expressed in midbrain dopaminergic cells.

In the rat, calbindin was detected in a high proportion of dopaminergic VTA cells and RRA cells, whereas in the SNl calbindin was mainly found in nondopaminergic neurons (Gerfen et al., 1985). In particular, as mentioned earlier (see Section 2.4), calbindin immunoreactivity (Figs. 4D; 13C,D) represents a distinctive chemoarchitectonic feature for the subdivision of dopaminergic midbrain neurons into dorsal (calbindin-positive) and ventral (calbindin-negative) tiers.

Some evidence suggests that midbrain dopaminergic neurons which contain calbindin are less vulnerable to neurotoxic insults (e.g. Liang et al. (1996) for data in the mouse) and to neurodegeneration in Parkinson's disease (see Lewis and Sesak, 1997). Calbindin-containing dopaminergic neurons are also spared in the weaver mutant mice (Gaspar et al., 1994), in which the DA neurons degenerate spontaneously.

Calbindin-immunopositive fibers densely innervate the SNr, and derive from calbindin-containing neurons of the matrix compartment of the striatum (Gerfen et al., 1985) (see Section 5.1).

Dopaminergic cells (identified with TH immunoreactivity) of the VTA, SNc, SNl, and of the caudal portion of the SNr contain calretinin (Rogers, 1992; Isaacs and Jacobowitz, 1994). In the rat, about 50% of midbrain dopaminergic neurons exhibit calretinin immunoreactivity. It is interesting to note that, in contrast to calbindin, calretinin is found in neurons of both the dorsal and ventral tiers.

Calbindin-immunoreactive and calretinin-immunoreactive neurons project to the frontal cortex and striatum (Gerfen et al., 1987). In the rat, the efferents of calbindin-positive neurons take part in the neostriatal mosaic (see Section 5.1), since they project selectively to the matrix compartment of the striatum, whereas calbindin-negative neurons innervate preferentially the patch compartment (Gerfen et al., 1985, 1987).

In the mouse, calbindin and/or calretinin expression in mesencephalic dopaminergic neurons was found to have a distribution similar to that reported in the rat, except for a less frequent colocalization of TH with either of these calcium binding proteins in the SNc and in the dopaminergic cells located in the SNr (Liang et al., 1996). Calbindin and calretinin were found in similar proportions in VTA and medial SNc neurons, suggesting that in the mouse the medial SNc portion may represent part of the A10 cell group rather than of the A9 cell group (Liang et al., 1996).

3.4. NEUROPEPTIDES

Neuropeptides are expressed in cell bodies, fibers and axon terminals in the VTA and in all the subdivisions of the SN, and are a main component of the chemical repertoire of the input–output organization of the midbrain dopaminergic system.

Approximately one-third of midbrain dopaminergic neurons contain the peptide cholecystokinin (CCK); these cells were initially identified in rat and man, mainly in the VTA, and were also seen in the SNc and SNl (Hökfelt et al., 1980a). This study, which was

based on sequential TH and CCK immunofluorescence, provided the first evidence of the coexistence of a neuropeptide with DA in neurons.

By means of immunofluorescence analyzed in the adjacent sections incubated with antibodies to CCK and TH, respectively, in combination with fluorescent retrograde tracing, Hökfelt and coworkers (1980b) could establish also the coexistence of TH and CCK in terminal fiber networks in the NAc and other targets of the mesolimbic system, and could determine that VTA neurons which contain both CCK and DA project to the caudal and medial portions of the NAc.

A large proportion of dopaminergic neurons in the rat VTA and medial SNc also contain the peptide neurotensin (Hökfelt et al., 1984b). In the rat, CCK is colocalized with neurotensin in more than 90% of neurotensin-positive neurons, whereas only 10–15% of the CCK-positive neurons contain neurotensin (Seroogy et al., 1989). Dopaminergic neurons of the ventral midbrain which contain CCK, or neurotensin or both are part of the mesolimbic and mesocortical systems (see Sections 7 and 8), since they project to the NAc, prefrontal cortex and amygdala (Seroogy et al., 1987). In the rat, the neuronal cell population that contains the peptides CCK and neurotensin also expresses the calcium binding protein calbindin (German and Liang, 1993).

The dendrites and axon terminals of midbrain dopaminergic neurons are endowed with CCK and neurotensin receptors (see for review Kalivas, 1993). As emphasized by Smith and Kieval (2000), such findings indicate that these neuropeptides can modulate the spontaneous activity or DA-containing neurons and/or control DA release in their targets.

Fiber terminal networks containing substance P, enkephalin and dynorphin are densely distributed in the ventral midbrain tegmentum (reviewed by Fallon and Loughlin, 1985, 1995). Terminal fibers containing substance P and those containing dynorphin are very dense in the SNr, and more sparsely distributed in the VTA and SNc, whereas the terminal fibers containing enkephalin are concentrated in the SNc and in the dorsal portion of the VTA.

These neuropeptides are coexpressed with GABA in subpopulations of striatal medium-sized spiny neurons, and therefore in subsets of the fibers which target the SN. As will be mentioned further (see Section 4.3), the striatal output reaches the SN through two main circuits distinct from the anatomical and functional points of view, defined as direct and indirect pathways. Direct pathway striatal neurons express the neuropeptide substance P and dynorphin, whereas indirect pathway striatal neurons express enkephalin (see Section 6.1).

The recently identified innervation of the SNc-VTA region by fibers which contain the peptide hypocretin/orexin is dealt with separately below, in view of its potential implication in distinct functions such as the regulation of arousal.

3.5. OREXIN/HYPOCRETIN-CONTAINING INNERVATION OF MIDBRAIN DOPAMINERGIC CELL GROUPS AND THEIR INVOLVEMENT IN STATE-DEPENDENT BEHAVIOR

Enhanced dopaminergic neurotransmission can influence both the sleep-wake cycle and the alternation of rapid eye movement (REM) and nonREM phases during sleep (see for review Pace-Schott and Hobson, 2002). The magnitude of the effect of DA on sleep cycles can also be argued on the basis of the potent effect of common psychostimulants (which

are inhibitors of DA reuptake) on the enhancement of wakefulness and prevention of sleep. Although the effect of dopaminergic drugs on sleep is beyond the scope of the present chapter, it is worth recalling here some features of the circuits which play a role in these mechanisms because of their interaction with the midbrain dopaminergic cells.

Cholinergic and aminergic cell groups of the brain stem, as well as cholinergic basal forebrain neurons, are key structures in the regulation of cortical activity, directly and through the thalamocortical system, resulting in the electroencephalographic synchronization and desynchronization which characterize slow-wave sleep, and wakefulness and REM sleep, respectively (see Steriade, 2003). Other key stations in these circuits are located in the hypothalamus, and are represented by the sleep-promoting ventrolateral preoptic nucleus of the anterior hypothalamus, and the wake-promoting tuberomammillary nucleus of the posterior hypothalamus (Saper et al., 2001; Steriade, 2003). Neurons of the tuberomammillary nucleus contain histamine, and give origin to fibers widely distributed in the brain; the histaminergic arousal system is modulated by influences of the aminergic brain stem cell groups (Haas and Panula, 2003). As mentioned in Section 3.1, midbrain dopaminergic cell groups are innervated by serotonergic fibers and interact with the noradrenergic system. Dopaminergic neurons of the SN and VTA have instead weak connections with the histamine-containing neurons of the tuberomammillary nuclei (Haas and Panula, 2003).

The firing rate of histaminergic, noradrenergic and serotonergic neurons decreases from nonREM to REM sleep, whereas firing of midbrain dopaminergic neurons does not seem to vary in phase with the REM–nonREM alternation during sleep and with the sleep/wake cycle (Miller et al., 1983). These data led to suppose that the effect of DA on sleep may be mediated by its interactions with other neurotransmitter systems (Pace-Schott and Hobson, 2002).

The understanding of the interaction of dopaminergic pathways with brain systems subserving state-dependent behavior has now received new clues from the finding that the midbrain dopaminergic cell groups are densely innervated by fibers containing orexins/hypocretins. These peptides (two products of a single gene, *Hcrt*) were described in 1998 by two different groups of investigators in a remarkable convergence of discoveries, which, however, has created some terminological complication because the peptides were baptized with two different names. In one study in which the gene for these molecules was cloned from the rat and the mouse, the peptides were called hypocretins because they are produced by hypothalamic neurons and have a weak homology to the gut peptide secretin (De Lecea et al., 1998). In the other study (Sakurai et al., 1998), the peptides were isolated with a chemical protocol in which brain extracts were used to stimulate a panel of orphan G-protein-coupled receptors. Sakurai et al. (1998) denominated the peptide orexin (from the Greek 'orexis', appetite) because their study ascertained the peptide activity in the control of food intake. Sakurai et al. (1998) also cloned the peptide cognate receptors: the orexin 1 receptor was shown to bind preferentially hypocretin 1/orexin A, whereas the orexin 2 receptor was shown to bind also hypocretin 2/orexin B with high affinity.

The neurons containing orexin/hypocretin are located in the dorsolateral and posterior hypothalamus and have widespread projections in the brain and in the spinal cord (Peyron et al., 1998), indicating that they may be involved in multiple functions (see the reviews by Kilduff and Peyron, 2000; Sutcliffe and de Lecea, 2002). In particular, the orexin/hypocretin system has been implicated in neuroendocrine and autonomic functions, in addition to food intake regulation. The hypocretin/orexin peptides are excitatory.

It has been ascertained in dogs, rodents and humans that narcolepsy, a disease characterized by the intrusion of REM sleep episodes into daytime wakefulness accompanied by loss of muscle tone, is associated with orexin ligand and receptor mutations or loss of orexin-producing neurons (reviews in Kilduff and Peyron (2000); Sutcliffe and de Lecea (2002); see also Sutcliffe and de Lecea (2004)). On the basis of this and other evidence, the orexin/hypocretin-containing cell group and their projections are considered to play a key role in arousal state control. The orexin/hypocretin-containing neurons exert an excitatory effect on tuberomammillary cells directly and by disinhibition (Eriksson et al., 2004), and may promote arousal through excitation of the other 'wake-active' monoaminergic cell populations which include noradrenergic and serotonergic neurons (Baldo et al., 2003), as well as via a link with midbrain dopaminergic neurons (see Kilduff and Peyron, 2000).

In the context of the present chapter, it is of special interest that orexin/hypocretin-containing hypothalamic neurons innervate in the ventral midbrain tegmentum the VTA and the SNc with a dense plexus of terminal fibers, whereas the SNr is substantially spared (Fig. 15). Consistently with this pattern of orexin/hypocretin innervation, in the rat orexin 1 and orexin 2 receptor mRNAs are very dense in both the VTA and the SNc, whereas no orexin receptor expression has been detected in the SNr (Marcus et al., 2001).

These findings open new perspectives for the understanding of the action of endogenous DA on state-dependent behavior, as well as of the effects of dopaminergic drugs on sleep and wake regulation, and other functions regulated by hypothalmic networks.

3.6. NITRIC OXIDE

The gaseous free radical nitric oxide (NO), a non-conventional neural messenger, is synthesized in neurons by the enzyme nitric oxide synthase (NOS), which can be revealed in histological sections by NADPH-diaphorase histochemistry or NOS immunohistochemistry. The role of NO in neural signaling has raised considerable interest (see, for example, Schmidt and Walter, 1994), stemming also from the finding that NOS has a discrete distribution in subsets of brain neurons, including intense expression in neuronal subsets of the striatum (Vincent, 2000).

Although NO modulation of basal ganglia circuits could mainly occur through release from striatal neurons and not at the level of midbrain dopaminergic cells, this free radical is mentioned here in view of its relevance in electrophysiological studies. It has been shown that NO affects burst firing induced in dopaminergic neurons by N-methyl-D-aspartate (Cox and Johnson, 1998). In addition, recent data have pointed out an important role of NO in indirect glutamate-mediated excitation of VTA neurons by nicotine (Schilström et al., 2004a,b). However, the mapping of NOS-containing neurons in the CNS did not point out histochemical positivity to the enzyme in dopaminergic cells (Vincent, 2000). On the other hand, a detailed study of NOS histochemical positivity in monoaminergic neurons of the rat brain (Johnson and Ma, 1993) has reported the occurrence of some NOS-positive neurons close to the mesencephalic midline and in the rostrodorsal VTA. In the same study (Johnson and Ma, 1993), the sequential staining for NOS and TH indicated that these enzymes were colocalized in less than 1% of the neurons positive to either marker. Thus, such data do not provide ground for a production of NO by dopaminergic cells, but this free radical could affect VTA neurons through diffusion from neighboring sources, which remains a subject for future investigations.

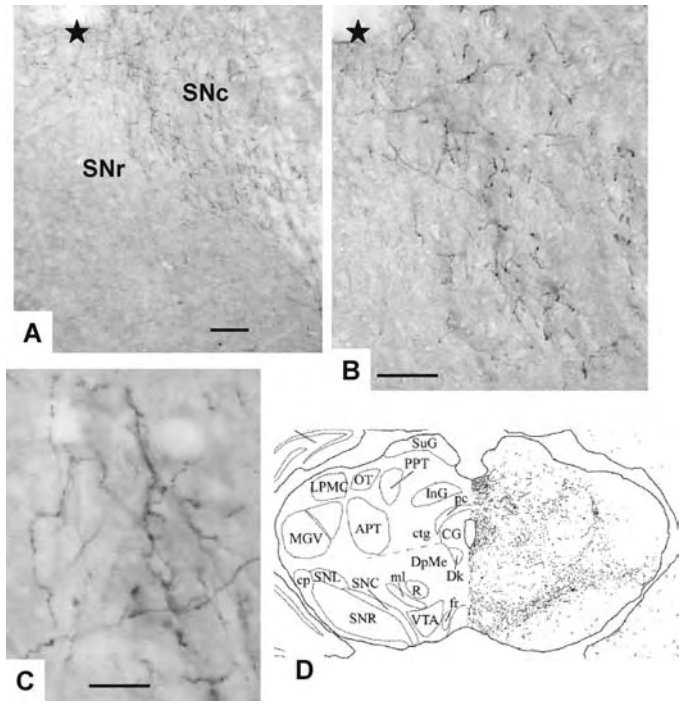

Fig. 15. The plate illustrates the orexin/hypocretin-containing innervation of the substantia nigra in the rat midbrain. Images A–C show orexin/hypocretin immunoreactivity (obtained with rabbit polyclonal antibody raised against orexin A from Santa Cruz Biotechnology, Santa Cruz, CA). Note in A the dense plexus, with varicose and beaded fibers, distributed throughout the pars compacta of the substantia nigra and sparing the pars reticulata. B represents at higher magnification an area of A; the star marks the same blood vessel for spatial reference. C shows details of the preterminal and terminal elements in the pars compacta from an adjacent section. Scale bars are equivalent to 75 μm in A, 20 μm in B, 10 μm in C. (A. Sadki and M. Bentivoglio). D is a schematic drawing of the hypocretin/orexin immunoreactivity in the rat midbrain (obtained with the antibody #250 from Sigma – St Louis, MO – raised against the 17-C terminal amino acids of hypocretin), reproduced with permission from Peyron et al. (1998). Abbreviations: APT, anterior pretectal nucleus; CG, central gray; cp, cerebral peduncle; ctg, central tegmental tract; Dk, nucleus Darkschewitsch; DpMe, deep mesencephalic nucleus; fr, fasciculus retroflexus; InG, intermediate gray layer of the superior colliculus; LPMC, lateral posterior thalamic nucleus, mediocaudal part; MGV, ml, medial lemniscus; OT, nucleus of the optic tract; PPT, posterior prectectal nucleus; R, red nucleus; SNC, SNc, substantia nigra, pars compacta; SNR, SNr, substantia nigra, pars reticulata; SNL, substantia nigra, pars lateralis; SuG, superficial gray layer of the superior colliculus; VTA, ventral tegmental area.

3.7. CONSTITUTIVE EXPRESSION IN MIDBRAIN DOPAMINERGIC NEURONS OF MOLECULES IMPLICATED IN NEURAL-IMMUNE INTERACTIONS

In the framework of neuroinflammatory mechanisms implicated in the neurodegenerative phenomena (see Section 2.6), and in particular in those affecting midbrain DA-containing neurons, growing interest is raised by the constitutive expression in the brain of molecules playing a role in these processes. These findings led to the hypothesis that immune molecules induced in pathological conditions could also act as modulators of neuronal activity in the normal brain (see, for example, the recent review of Boulanger and Shatz, 2004).

In relation to this, it should be recalled that the CNS has long been considered an immune-privileged site. This assumption was also based on the fact that the expression of major histocompatibility complex (MHC) molecules was considered to be low or absent in normal conditions. However, expression of MHC molecules can be induced in neurons and glia after different kinds of insult (see, for example, Fabry et al., 1994). In addition, constitutive expression of MHC class I genes has been detected in the rodent brain (Lidman et al., 1999; Boulanger and Shatz, 2004). MHC class I genes, which present peptides to CD8+ immunocompetent T cells, consist of classical, Ia, and nonclassical, Ib, types, sharing varying degree of homology. In the study of Lidman et al. (1999) most prominent expression of a set of MHC class Ib genes named RT1-U was detected in the rat SNc neurons. In addition, in the adult rat brain stem dopaminergic SNc neurons were found to express high levels of MHC class I heavy chain mRNA, as well as mRNA for β2-microglobulin, a light chain molecule noncovalently bound to MHC class I heavy chain for functional presentation of antigenic peptides to CD8+ T cells (Linda et al., 1999). In the same study, also dopaminergic VTA cells were found to express both the above mRNAs (whose expression was instead very low in the SNr), but at much lower levels than in the SNc. MHC class 1 mRNA, and in particular the mRNA for the H2-D MHC class I molecule, was also found to be highly expressed in the SNc of the adult mouse brain (see Boulanger and Shatz (2004) and Fig. 14D). Altogether these findings recall attention on the potential involvement of immune-related molecules in the activity of midbrain dopaminergic cells and have potential implications for the involvement of these cells in disease, and in particular in neurodegenerative conditions such as Parkinson's disease.

Special attention has also been devoted in recent years to the expression of chemokines (a term originally introduced to describe a family of chemoattractant cytokines) in the CNS (Asensio and Campbell, 1999; Bacon and Harrison, 2000; Bajetto et al., 2001). Chemokines are low molecular weight soluble proteins, classified in different subgroups. Through G-protein-coupled cell-surface receptors, chemokine activities mediate a variety of biological activities, and especially leukocyte responses including chemotaxis and immune activation. On the basis of the constitutive expression of some chemokines and chemokine receptors in brain neuronal subpopulations and glial cells, these molecules are now implicated also in physiological mechanisms in the developing and mature CNS. Although the functional significance of the constitutive expression of chemokines and their receptors in the CNS is still poorly understood, such mechanisms include neuronal patterning and migration during development, as well as synaptic transmission and plasticity in adulthood.

In studies devoted to the immunohistochemical identification of cells which express constitutively chemokines and their receptors, the distribution of the chemokine stromal cell-derived factor 1 (SDF-1/CXCL12) was found to be highly regionalized, and its expression was detected in dopaminergic cells of the VTA and SNc, as well as in SNr cells (Banisadr et al., 2003). In this study, SDF-1/CXCL12 was identified in approximately 80% of neurons in the SNc. CXCR4, the cognate receptor of SDF-1/CXCL12, was also found to be highly expressed in dopaminergic cells of the VTA and the SNc (whose phenotype was confirmed by TH immunoreactivity), and to a lesser extent in the SNr (Banisadr et al., 2002a). Expression of another chemokine receptor, CCR2, which is the receptor for the monocyte chemoattractant protein-1/CCL2, was also detected in the SN with reverse transcriptase-polymerase chain reaction (RT-PCR) and receptor binding, and predominated in the dorsal tier of the SNc and ventrolaterally in the SNr (Banisadr et al., 2002b). In these studies, the expression of chemokines and chemokine receptors was

detected in different brain regions, and it is indeed intriguing that midbrain dopaminergic cell groups are among the sites of a potential neuromodulatory function of these molecules in the normal brain.

4. NEURAL WIRING IN THE BASAL GANGLIA

4.1. 'EXTRAPYRAMIDAL SYSTEM', AND BASAL GANGLIA COMPONENTS

In order to discuss the organization of dopaminergic pathways, an overview of basal ganglia components and circuits is first presented. These circuits form the system that was traditionally defined as 'extrapyramidal' and is now indicated with the more straightforward definition of 'basal ganglia'.

The term 'extrapyramidal system' has exerted a high impact in the clinical and basic neuroscience of the 20th century. It is commonly believed that this term was introduced by Wilson in 1912, but Parent (1986) noted that it was actually first used at the end of the 19th century by Prus (1898), when terms such as 'extrapyramidenbahnen' (extrapyramidal tracts) were commonly employed by the members of the Vienna school of neurology dominated by Meynert. The adjective 'extrapyramidal' is still widely used in clinical neurology for the definition of symptoms and syndromes caused by basal ganglia dysfunction. However, as pointed out by Nauta (1989) the term 'extrapyramidal system' has never been satisfactorily defined anatomically. The designation of 'basal ganglia' is therefore more helpful in facilitating communication between neuroscientists. Although, acceptable from the functional point of view, the definition of basal ganglia (literally indicating the gray matter structures located at the base of the cerebral hemispheres) is not equivalent, from the classical anatomical point of view, to the structures whose alterations cause movement disorders. Therefore, in anatomy textbooks a variety of telencephalic structures, including the claustrum and the amygdala, may be designated collectively as basal ganglia.

The main structures of the basal ganglia, defined nowadays in neuroscience as a system of functionally related and anatomically interconnected centers and circuits, include the striatum, the GP, the STh, and the SN (Fig. 16). The 'umbrella term' of basal ganglia therefore groups structures located in the telencephalon (the striatum and GP), diencephalon (the STh) and brain stem (the midbrain dopaminergic cell groups).

The striatum comprises the caudate nucleus and the putamen (the 'neostriatum', frequently indicated, as in this chapter, simply as 'striatum') and the ventral striatum. The striatal components represent the key regions for DA release and action in the basal ganglia, and are dealt with in Sections 5 and 7.

The GP, recognized as an individual anatomical entity by Burdach (see Parent, 1986), lies lateral to the internal capsule. In primates, the GP appears paler than the adjacent striatum in Nissl-stains (hence its definition as 'pallidus'), and is divided by the internal medullary lamina into a lateral or external segment (GPe) and a medial or internal segment (GPi) (Fig. 16). In most nonprimate mammalian species, neurons which form the GPi are completely surrounded by fibers of the internal capsule, thus forming the entopeduncular nucleus (EP), a structure homologous to the GPi in primates, whereas the term 'globus pallidus' refers to the division homologous to the GPe. These terminological differences are, however, moving at present towards a simplification (sometimes even neuroanatomists make an effort to simplify terminologies and homologies). Therefore, the

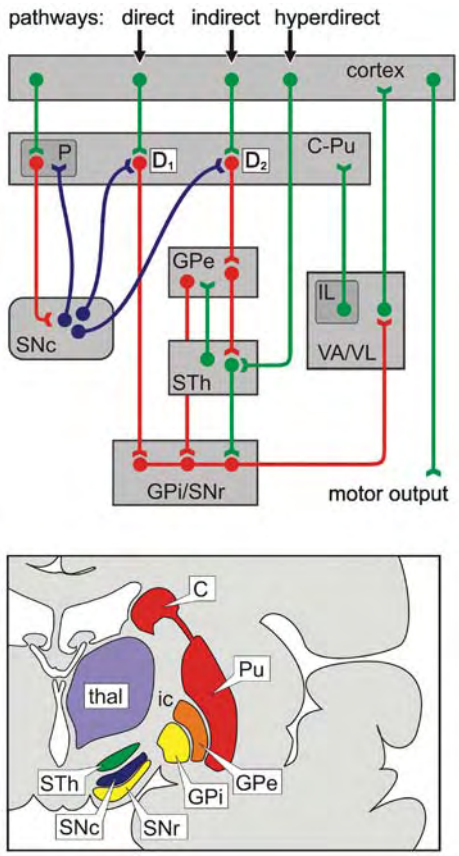

Fig. 16. The diagram summarizes the main neural circuits which subserve the processing of neural information in the basal ganglia, whose key centers are shown below in the outline of a section through the primate brain. In the diagram, excitatory projections are in green, inhibitory projections in red and the dopaminergic input to the striatum in blue. For a simplification, not all the connections have been indicated. In the anatomical scheme, the striatum is in red, and output nuclei of the basal ganglia (the internal segment of the globus pallidus, GPi, and the substantia nigra pars reticulata, SNr) are in yellow. The striatum (comprising the caudate, C, and the putamen, Pu) receives three major inputs: corticostriatal projections; thalamostriatal projections deriving primarily from the intralaminar nuclei (IL); dopaminergic projections deriving from the midbrain and in particular from the substantia nigra pars compacta (SNc), which reach both the striatal patch compartment (P) and the matrix. The basal ganglia output nuclei convey information to the thalamus, targeting in particular the ventral tier of thalamic nuclei (the ventral anterior and ventral lateral nuclei of the thalamus, VA/VL), which project via the thalamocortical system to frontal cortical areas giving origin to cortical descending pathways. Through the direct pathway of information processing, striatonigral and striatopallidal neurons reach directly the basal ganglia output nuclei. In the indirect pathway, different subsets of striatal neurons reach the basal ganglia output nuclei through a relay in the external segment of the globus pallidus (GPe), interconnected with the nucleus subthalamicus (STh) which, in turn, provide excitatory inputs to the basal ganglia output nuclei. In addition, through the hyperdirect pathway the STh is regulated directly by cortical input bypassing the striatum. Striatal cell populations of the direct and indirect pathways are distributed in the matrix compartment of the striatum. Direct pathway striatal neurons bear preferentially D_1 dopamine receptors and indirect pathway neurons bear preferentially D_2 receptors. A different population of striatonigral neurons in the patch compartment of the striatum (P) projects to the SNc. Dopaminergic efferents of midbrain cell groups also innervate directly the GPe and the STh (not shown in the diagram; see Section 6.2 of the text). Other abbreviations: ic, internal capsule; thal, thalamus.

EP is frequently defined nowadays as GPi (or medial GP) also in rodents, adopting in rodents the subdivision of the GP in two segments (cf. the rat atlases of Paxinos and Watson (1998) and Swanson (1992); cf. also the mouse atlases of Hof et al. (2000) and Paxinos and Franklin (2001)). The majority of neurons of both the GP divisions are large and fusiform or triangular, with very long, thick, smooth and sparsely branching dendrites (see for reviews Heimer et al., 1995, and Gerfen, 2004). The term ventral pallidum refers to the ventral or subcommissural part of the pallidal complex (see Section 7).

The STh was described by Luys (1865), and was designated by Forel in 1877 as 'corpus Luysii' (Pearce, 2003). The STh is an ovoid nucleus bordered dorsally by the zona incerta and medially by the lateral hypothalamus. Relatively prominent in the rat, the STh contains densely packed, medium sized, fusiform or polygonal neurons, giving off relatively long dendrites with a few or moderate number of spines. In the rat, the dendritic field of a STh neuron usually covers the whole extent of the nucleus and occasionally crosses its borders (see for review Heimer et al., 1995). The role of the STh in basal ganglia circuitry has recently received considerable attention as a target structure for stereotaxic surgery in Parkinson's disease, and will be discussed (Section 4.3) in the context of information processing in the basal ganglia.

4.2. OVERVIEW OF BASAL GANGLIA CIRCUITRY

Basal ganglia circuits have unique features in the brain, related to the abundance of feed-forward loops of information processing. In general terms, neural information is funneled into the striatum, the main input region to the basal ganglia, from three main sets of afferents: the corticostriatal, thalamostriatal and nigrostriatal pathways. Information is then processed within basal ganglia circuits, and exits from the basal ganglia to be conveyed to the thalamus and channeled from there mainly to the cortical fields, i.e. a cortical region far more restricted than those from which the information departed. In addition, the basal ganglia output is conveyed to the brain stem centers, including the superior colliculus and the pedunculopontine nucleus in the mesopontine tegmentum. Therefore, as summarized by Graybiel (1990), the basal ganglia collect signals from the cerebral cortex, 'redistribute these cortical inputs both with respect to one another and with respect to inputs from the limbic system, and then focus the outputs of these redistributed, integrated signals to particular regions of the frontal lobes and brainstem involved in aspects of motor planning and motor memory'.

The main circuits of the basal ganglia are summarized in Fig. 16. Corticostriatal fibers, which derive from Layer V of nearly the entire cortical mantle, are glutamatergic and excitatory. The terminal fields of the different sets of cortical projections determine in the striatum distinct anatomofunctional regions (see below).

As for the two additional channels of information destined to the striatum, dopaminergic fibers ascending from the ventral midbrain tegmentum are part of the basal ganglia loops. The other main input derives from the thalamus, and mainly (but not exclusively) from the intralaminar nuclei. Both the anterior intralaminar central lateral and paracentral nuclei, and the posterior intralaminar structures (represented in rodents by the parafascicular nucleus, which expands in carnivores and primates in the center median-parafascicular complex) give origin to thalamostriatal projections. As the cortical fibers, thalamostriatal fibers utilize an excitatory amino acid as neurotransmitter, and their termination is compartmentalized in the striatum (see Section 5.1).

The functional significance of the thalamostriatal innervation, which equals in density, the corticostriatal and nigrostriatal inputs, has been less studied than that of the other two channels of striatal input. Although a discussion of this problem goes beyond the scope of the present chapter, it is worth mentioning that the intralaminar nuclei, and the midline nuclei which are the main source of thalamostriatal inputs terminating in the NAc (see Section 7.1) are the structures grouped under the so-called 'nonspecific thalamus'. This thalamic region was supposed to give origin to widespread cortical and subcortical projections, and has been traditionally implicated in the activation of cortical activity as a relay of ascending brain stem pathways (see Bentivoglio et al., 1991; Groenewegen and Berendse, 1994; Steriade, 2003). It has, however, become clear that the intralaminar nuclei are also inserted in parallel processing in basal ganglia-thalamocortical circuits (Groenewegen and Berendse, 1994; O'Donnell et al., 1997). The mysterious role of the thalamostriatal system has been recently examined in the monkey (Matsumoto et al., 2001). This latter study pointed out that neurons of the center median-parafascicular complex supply striatal neurons with information about attention-demanding, behaviorally significant sensory events, which can activate conditional responses of striatal neurons in combination with dopaminergic inputs having motivational value.

The striatum is the major target of midbrain dopaminergic neurons. By reaching the striatal complex, DA acts as protagonist of basal ganglia circuits modulating striatal cells via the DA receptors. In particular, DA regulates the activity of the striatal neurons of the so-called direct and indirect pathways of basal ganglia processing of cortical information (see Section 4.2). Through these pathways, information is conveyed to the output nuclei of the basal ganglia, the SNr and the GPi, via the GABAergic striatonigral and striatopallidal projections. Dopaminergic fibers also innervate the GP and the STh (see Section 6.2), thus modulating directly extrastriatal targets, and influencing the activity of STh neurons in the so-called hyperdirect pathway which bypasses the striatum.

In addition, a set of striatonigral fibers project to the SNc, establishing a reciprocal loop with the midbrain dopaminergic neurons. Another main loop within the basal ganglia is represented by the circuit linking the GP and the STh, inserted in the indirect pathway of basal ganglia processing. STh neurons are glutamatergic and excitatory, and studies in the rat have indicated that they are highly collateralized, giving off axon collaterals to both pallidal divisions and to the SNr (although this collateralization is still disputed in primates).

The information finally exits from the basal ganglia conveyed by GABAergic efferents of the ouput nuclei. The SNr gives origin to the nigrothalamic pathway, and the GPi to the pallidothalamic pathway (contained in the ansa lenticularis and lenticular fasciculus). The SNr and GPi are the two 'Ambassadors' (which can also be viewed as 'Ministers of Foreign Affairs' of the basal ganglia kingdom) which communicate to the thalamus messages processed in the basal ganglia. The main target of the basal ganglia output is the ventral tier of thalamic nuclei, from which information reaches frontal cortical areas through thalamocortical pathways. The pallidothalamic and the nigrothalamic pathways also reach the posterior intralaminar nuclei, configurating an internal loop of the basal ganglia because these structures, as mentioned above, give origin to thalamostriatal fibers. The intralaminar nuclei, however, project also to the cerebral cortex, and in particular to the frontal cortical areas (Macchi and Bentivoglio, 1986); these thalamocortical fibers are in part represented by collaterals of thalamostriatal fibers (Macchi et al., 1984).

Similar to all the other thalamic afferent inputs, basal ganglia outputs give off collaterals to the thalamic reticular nucleus traversing this nucleus to enter the thalamus

(Smith et al., 1998). The thalamic reticular nucleus is an inhibitory sheet of GABAergic neurons, which belong to the ventral thalamus and surround the nuclei of the dorsal thalamus. The reticular nucleus projects to thalamic nuclei, playing a role as pacemaker of the excitatory activity of thalamic relay neurons (see Steriade, 2003). Therefore, GABAergic neurons of the thalamic reticular nucleus provide an additional gate for the final functional outcome of basal ganglia output on thalamocortical neurons. This gate plays a role in the information processing pathways deriving from both the neostriatum (Smith et al., 1998) and the ventral striatum (O'Donnell et al., 1997).

In the cortex and from the cortex, information is conveyed through corticocortical and descending pathways. In particular, information meets in the cortex the sites of origin of the descending motor pathways, including cortical-brain stem pathways and the corticospinal tract.

As already mentioned, basal ganglia efferents also reach directly the brain stem (see the reviews by Alexander and Crutcher, 1990; Smith et al., 1998). The GPi projects to the pedunculopontine nucleus, which is also innervated by the SNr. Cholinergic and noncholinergic neurons of the mesopontine tegmentum reciprocate input to the basal ganglia, projecting to different components, including the SN (see Section 3.1). The SNr sends projections to the superior colliculus via the GABAergic nigrotectal pathway. This pathway, which is largely formed by collaterals of the nigrothalamic pathway (Bentivoglio et al., 1979), is considered to play a key role in visuomotor integrative functions.

The rodent motor thalamus consists of two main components: the ventromedial nucleus and the ventral anterior/ventral lateral nuclear complex. In the rat, both components are targeted by nigrothalamic fibers and by pallidothalamic fibers arising from both the pallidal divisions, and are conveyed to the medial agranular cortex, equivalent to the primate supplementary motor and premotor areas (Sakai et al., 1998; Kha et al., 2000; Sakai and Bruce, 2004).

It should also considered that in the rat the projections of the thalamic ventromedial nucleus are widely distributed upon the cortical layer I (Herkenham, 1979). Despite the differences in the rodent and the primate motor thalamus and thalamocortical systems, a similar organization may also occur in primates. In the monkey, wide cortical projections to the most superficial layer arising from the magnocellular portion of the ventral anterior nucleus, which is the nigrothalamic recipient territory, have been detected (Bentivoglio et al., 2000). Therefore, information processed in basal ganglia may exert an integrative effect on behavior not only through their connections with the motor system, but also by modulating the processing of sensory information across a wide expanse of the cerebral cortex.

4.3. THE DIRECT, INDIRECT AND HYPERDIRECT PATHWAYS OF BASAL GANGLIA INFORMATION PROCESSING

As emphasized by Smith et al. (1998), when a large amount of data was gathered about the anatomical and functional organization of the basal ganglia and the pathophysiology of movement disorders associated with diseases that affect this system, an effort was made at the end of the 1980s to formulate a unifying model of the functional organization of the basal ganglia accounting for both normal and abnormal function (Albin et al., 1989). This model was rapidly elaborated and expanded (Alexander and Crutcher, 1990), also in view of the previous definition of parallel cortico-basal ganglia-thalamocortical circuits (Alexander et al., 1986), and is still the subject of extensive investigations

(see, inter alia, the reviews of Smith et al., 1998; Gerfen, 2000, 2004). The model is based on the so-called 'direct' and 'indirect' pathways, to which a 'hyperdirect' pathway was added (Nambu et al., 1996), for the flow of cortical information through the basal ganglia and the DA modulation.

According to this model (Fig. 16), cortical information conveyed to the striatum by corticostriatal afferents is processed in the striatum and transmitted to the output nuclei of the basal ganglia (the SNr and GPi) directly, through the inhibitory striatonigral and striatopallidal projections, or indirectly via the GP and the STh. Thus, the indirect pathway striatal neurons reach the SNr and GPi through a relay in the GPe, interconnected with the STh neurons which, in turn, provide excitatory inputs to the basal ganglia output nuclei. The STh is considered to play, together with the GPe, a role of central pacemaker (Plenz and Kitai, 1999) inserted in the indirect pathway. The dopaminergic modulation of these pathways will be discussed in Section 6.

The distinct striatal projection pathways contribute differentially to the excitatory and inhibitory circuits regulating the basal ganglia output, resulting in functionally opposite effects: the direct pathways lead to a disinhibition of the target regions, whereas the indirect pathways lead to their inhibition. Therefore, the roles of the direct and indirect pathways are implicated in the activation and suppression of motor behavior, respectively: activation of the direct pathway is thought to facilitate motor behavior, whereas the indirect pathway is thought to inhibit inappropriate motor behavior.

The STh receives also direct excitatory input from the cerebral cortex, especially from the frontal cortical areas: the primary motor cortex, with a minor contribution of prefrontal and premotor areas (see for review Nambu et al., 2002). Cortical afferents from the primary motor cortex in rodents and cats are composed of collaterals of the pyramidal tract or of corticostriatal fibers (reviewed by Hamani et al., 2004). The hyperdirect pathway is a cortico-STN-pallidal pathway, which conveys excitatory input from the motor-related cortical areas to the GP bypassing the striatum, and therefore with shorter conduction time. According to the model of the hyperdirect pathway, the activity of the cortico-STh-pallidal route could result in a wide inhibitory effect on motor programs, with 'adjustments' of signals through the direct cortico-striato-pallidal pathway (Nambu et al., 2002).

Despite the emphasis on parallel pathways in this conceptual scheme, it should also be considered that information processing through the direct and indirect pathways is subserved by complex synaptic interactions: striatal neurons giving origin to the direct pathway are synaptically interconnected with indirect pathway striatal neurons, and the direct and indirect pathways converge at the synaptic level on single output neurons of the basal ganglia (Smith et al., 1998).

The development of this robust conceptual model of information processing in the basal ganglia and functional effects on the target regions has brought about important consequences also in clinical studies and in the development of new therapeutical approaches for the treatment of Parkinson's disease in humans. These are based on deep brain stimulation techniques, and in particular, on the electrical stimulation of the STh through chronically implanted electrodes, which was found to eliminate or alleviate resting tremor, rigidity and bradykinesia in Parkinson's disease (see, for example, Lozano et al., 2002; Benabid, 2003). In a remarkable interaction between the basic and the clinical neurosciences, these findings are, in turn, boosting research on the mechanisms underlying these therapeutical effects (Bevan et al., 2002; Surmeier and Bevan, 2003; Hamani et al., 2004).

4.4. DESCENDING EFFERENTS OF THE MIDBRAIN DOPAMINERGIC CELL GROUPS

The projections ascending to the forebrain are the main efferents of midbrain dopaminergic cells, which, however, give also origin to some descending projections. These efferents, which will be mentioned briefly here, can explain features of the distribution of DA receptors in the brain stem and cerebellum, which will be reviewed in the last part of this chapter.

In their study of the efferent connections of the SN and VTA performed in the rat with anterograde tracing using tritiated amino acids and autoradiography, Beckstead et al. (1979) detected little input to the brain stem. However, they could trace efferents to the central gray, mesopontine structures including the pedunculopontine tegmental nucleus, the dorsal raphe and median raphe nuclei, with a sparse innervation of the locus coeruleus. Dopaminergic axons originating in the SNc, VTA and medial hypothalamus have been described in subsequent studies to reach the mesencephalic trigeminal nucleus, with extension to the parabrachial nucleus and to the locus coeruleus (Copray et al., 1990; Maeda et al., 1994).

Until recently, the cerebellum was not considered to utilize DA as a neurotransmitter, and the DA present in the cerebellum was considered to serve only as a precursor for noradrenaline in afferent fibers supplied by the locus coeruleus. However, DA release and binding and dopaminergic innervation have been reported in the 1990s in the rodent cerebellum (Panagopoulos et al., 1991; Chrapusta et al., 1994), in which, as presented in the last part of this chapter, the presence of DA receptor subtypes has been repeatedly described. These findings motivated mapping studies based on tract tracing combined with TH immunohistochemistry in the rat (Ikai et al., 1992), and TH and DAT immunohistochemistry in the monkey (Melchitzky and Lewis, 2000).

Ikai et al. (1992) reported that the VTA sends projections to the rat cerebellar cortex and deep cerebellar nuclei bilaterally, with a slight contralateral predominance. In this study, dopaminergic efferents of the A10 cell group were reported to reach mainly the granule cell layer of the cerebellar cortex in the lateral portion of the hemispheres, with additional input to the Purkinje cell layer, but sparing the molecular layer. The deep cerebellar nuclei, and in particular the lateral nucleus, were instead found to receive inputs from nondopaminergic cells of the VTA, reciprocating projections to the VTA bilaterally and with a contralateral predominance.

In the monkey cerebellar cortex, Melchitzky and Lewis (2000) have recently described a dopaminergic innervation that matched the rat data in terms of laminar distribution (reaching mainly the granule cell layer and arborizing densely in the subjacent Purkinje cell layer), but was confined to certain lobules of the cerebellar vermis.

5. DOPAMINERGIC INNERVATION OF THE STRIATUM

5.1. THE STRIATUM, STRIATAL COMPARTMENTS AND FUNCTIONAL SUBDIVISIONS

Detected by anatomists who dissected the cerebral hemispheres, the main structure of the basal ganglia was defined as ‘corpus striatum’ by Thomas Willis in the 17th century (Willis, 1664) because of the mixture of gray matter and fiber tracts. Such mixture was

described as follows by de Vieussens (1684), an admirer of Willis who wished to advance Willis' work: '. . .[the white matter tracts] which have been so disposed that, mixed with the ashen substance [gray matter], they somewhat resemble bodies marked by striae' (translation provided by Clarke and O'Malley, 1996). However, as noted by Parent (1986), Vicq d'Azir (1786) was the first to realize that the caudate nucleus and the putamen belonged to the same structure defined as striatum.

In primates and in many nonprimate mammals, the striatum is divided by the internal capsule into the caudate nucleus, located dorsomedially, and the putamen, located ventrolaterally. In other mammalian species, including the rat and the mouse, the bundles of the internal capsule traverse the striatum 'in the form of a brush rather than a plate' (Nauta, 1989), and the striatum cannot, therefore, be subdivided in two entities, so that it is often referred to as caudoputamen or caudate-putamen (CPu).

As mentioned earlier, the striatum is classically divided into (*i*) dorsal striatum or neostriatum, which includes most of the caudate and putamen, and (*ii*) ventral striatum, which includes the NAc, the ventromedial parts of the caudate and putamen, and the striatal portion of the olfactory tubercle.

The principal neuronal cell type of the striatum is the medium spiny projection neuron. This cell type accounts for about 95% of the striatal neuronal population and is rather homogeneously distributed. Approximately half of these neurons project to the SNr and the other half to the GPe. In these neurons, GABA coexists with neuropeptides, providing a chemical signature of cell subpopulations of striatonigral and striatopallidal neurons (see Section 6.1), which are interspersed with one another. The remaining striatal neurons are interneurons; these include large aspiny neurons which utilize acetylcholine as neurotransmitter and medium aspiny neurons which utilize GABA as neurotransmitter. Cholinergic neurons receive a dopaminergic input, and acetylcholine release is under dopaminergic control, configurating complex interactions between these neurotransmitters and glutamatergic inputs in the control of the activity of striatal neurons (see, inter alia, Di Chiara et al., 1994; Nicola et al., 2000; West et al., 2003). The intrinsic organization of the striatum is beyond the scopes of the present chapter, and the reader is referred to recent extensive accounts in the rat (Gerfen, 2004), and in primates (Haber and Johnson Gdowski, 2004). It is worth mentioning, however, that different classes of chemically characterized interneurons can influence differential responses of the striosome (or patch) and matrix compartments of the striatum, thereby regulating the differential responses of striatal projection neurons to DA-mediated signaling (Saka et al., 2002).

An important characteristic of the dorsal striatum is its compartmental organization, based on the subdivision into patch/matrix compartments; in carnivores and primates, the patches are mostly termed as striosomes, which is their original definition. In fact, the compartments were first detected in sections processed for histochemistry to reveal acetylcholinesterase (AChE) activity (Graybiel and Ragsdale, 1978) as AChE-poor zones (the striosomes), embedded in the large AChE-rich tissue (the extrastriosomal matrix) of the human, monkey and cat striatum. These observations stimulated intense research activities in many laboratories. The findings brought about evidence for the compartmentalization of the vast majority of neuroactive molecules in the striatum (such as, neurotransmitters, neurotransmitter receptors and their binding sites, and a variety of neuromodulatory molecules and their receptors). These chemoarchitectural data were paralleled by data on striatal connectivity, so that all input-output connections of the striatum, as well as many intrinsic connections subserved by interneurons, were found to be organized following the patch/matrix compartmentalization (see for reviews Graybiel,

1990; Gerfen, 2004). The patch compartments of the striatum are characterized by low levels of acetylcholine and high levels of various opiates and substance P. The matrix compartment is characterized by cholinergic and somatostatin-containing neurons. Anatomically, corticostriatal and thalamostriatal projections are closely associated with the striatal matrix, while projections from limbic structures, such as the hippocampus and the amygdala, primarily innervate striatal patches.

In addition, and superimposed to the compartmental patch/matrix organization, corticostriatal projections determine a tripartite anatomical and functional subdivision of the striatum into motor, associative and limbic territories, which have been the subject of detailed investigations in both the monkey and the rat (reviewed by Joel and Weiner, 2000).

In the rat, the motor striatum comprises the lateral portion of the CPu and receives input from the motor cortex (lateral and medial agranular cortical fields). This region of the striatum is equivalent in primates to the dorsolateral portion of the caudate nucleus and the dorsolateral putamen caudal to the anterior commissure, which receives input from the primary motor, premotor and supplementary motor areas.

The associative striatum comprises instead in the rat the medial portion of the CPu, which receives input from the anterior cingulate area (considered analogous to the dorsolateral prefrontal cortex in primates). In primates the associative striatum includes most of the head, body and tail of the caudate and large parts of the putamen rostral to the anterior commissure; it receives input from associative areas of the cortex, including those of the prefrontal cortex.

In the rat, the limbic or ventral striatum, which includes the ventral striatum proper and the ventromedial portion of the CPu, receives extensive input from limbic structures, such as the hippocampus and amygdala, as well as prefrontal cortical areas subserving limbic and autonomic functions (orbital, infralimbic, prelimbic and agranular insular fields) (see Section 7.2). In primates, the limbic striatum comprises the NAc and the most ventral parts of the caudate and the putamen and, as in the rat, receives input from the hippocampus and amygdala; its cortical input is further defined by projections deriving from the orbitofrontal cortex and the anterior cingulate area.

5.2. THE NIGROSTRIATAL PATHWAY

Following the debates and observations summarized in Section 1.1, Lindvall and Björklund (1974b) traced the trajectory of the nigrostriatal pathway by means of the glyoxylic acid-histofluorescence technique (Lindvall and Björklund, 1974a). Ascending from the midbrain, nigrostriatal fibers traverse the Forel field and then course into the dorsolateral medial forebrain bundle (Veening et al., 1982). The fibers then run through the ventromedial edge of the internal capsule and enter the striatum by several routes.

Since this initial description, a wealth of data has increased knowledge on the organization of nigrostriatal projections (see, for example, the reviews by Smith and Kieval, 2000; Gerfen, 2004). Data on the organization of the nigrostriatal pathway have thus been obtained in relation to a number of features, including the topography of the cells of origin, pattern of axonal arborization, pattern of termination in striatal compartments, synaptic organization.

Neurons projecting to the striatum arise from all subdivisions of midbrain dopaminergic cell groups (Figs. 17, 18A), being distributed in the ventral midbrain tegmentum in a somewhat continuous manner (see, for example, the retrograde tracing

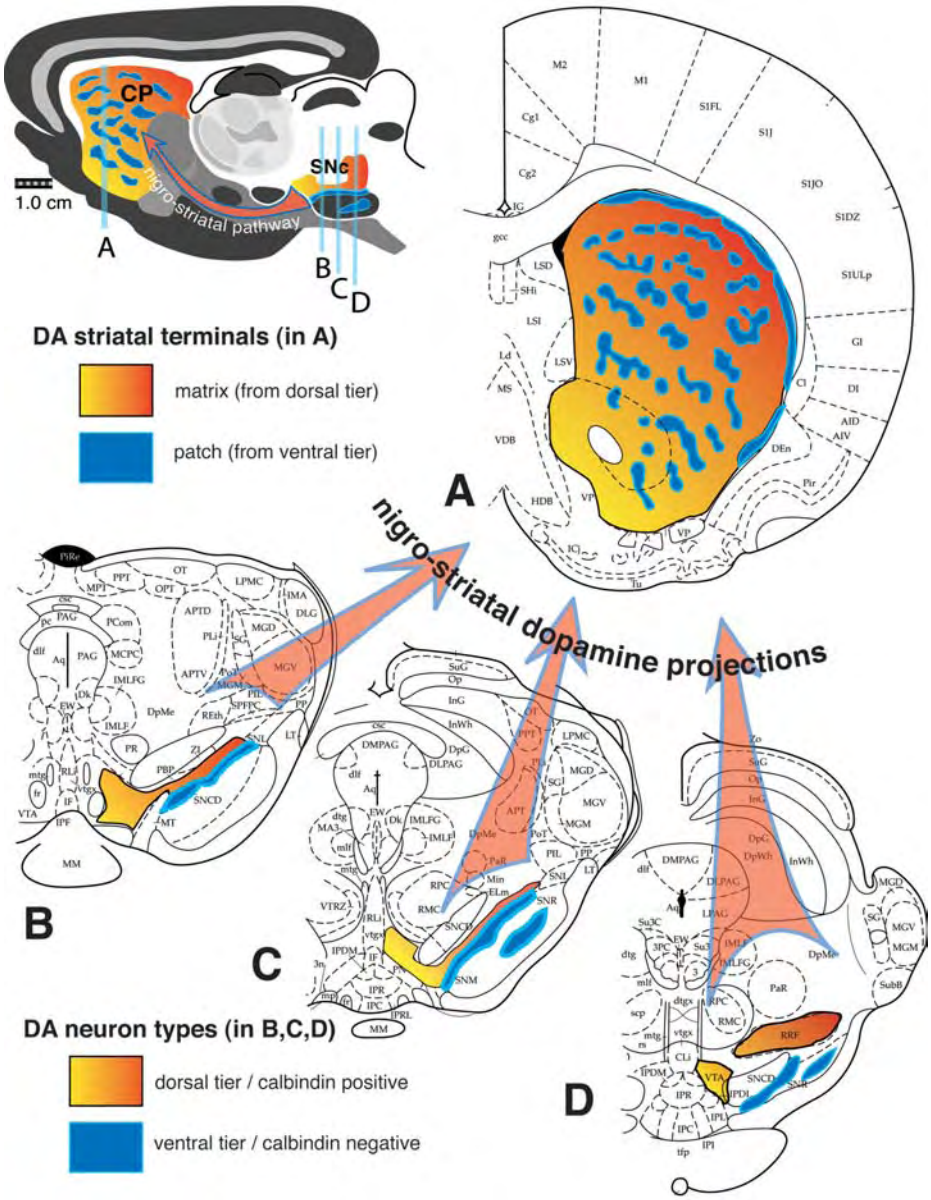

Fig. 17. The figure illustrates the organization of the nigrostriatal dopaminergic pathway (shown in the sagittal diagram at upper right) in the rat, in relation to the inputs to the patch and matrix compartments of the striatum deriving from the dorsal tier and the ventral tier, respectively, of midbrain dopaminergic neurons. The termination of dopaminergic axons in the striatal compartments is illustrated in a coronal section through the striatum (A, corresponding to the level A indicated in the sagittal figurine). Coronal sections through the midbrain (B–D, cut at the corresponding levels indicated in the sagittal figurine) illustrate the location of the dorsal and ventral tiers. A general topography is also shown, in that dopaminergic neurons located medially in ventral midbrain tegmentum project ventrally in the striatum, including the territory of the nucleus accumbens, whereas laterally located midbrain dopaminergic cells project to dorsal striatal regions. Abbreviations of structures of the ventral midbrain tegmentum and surrounding it can be found in the legend to Fig. 4; all the other abbreviations can be found in the rat atlas of Paxinos and Watson (1998). Reproduced with permission from Gerfen (2004).

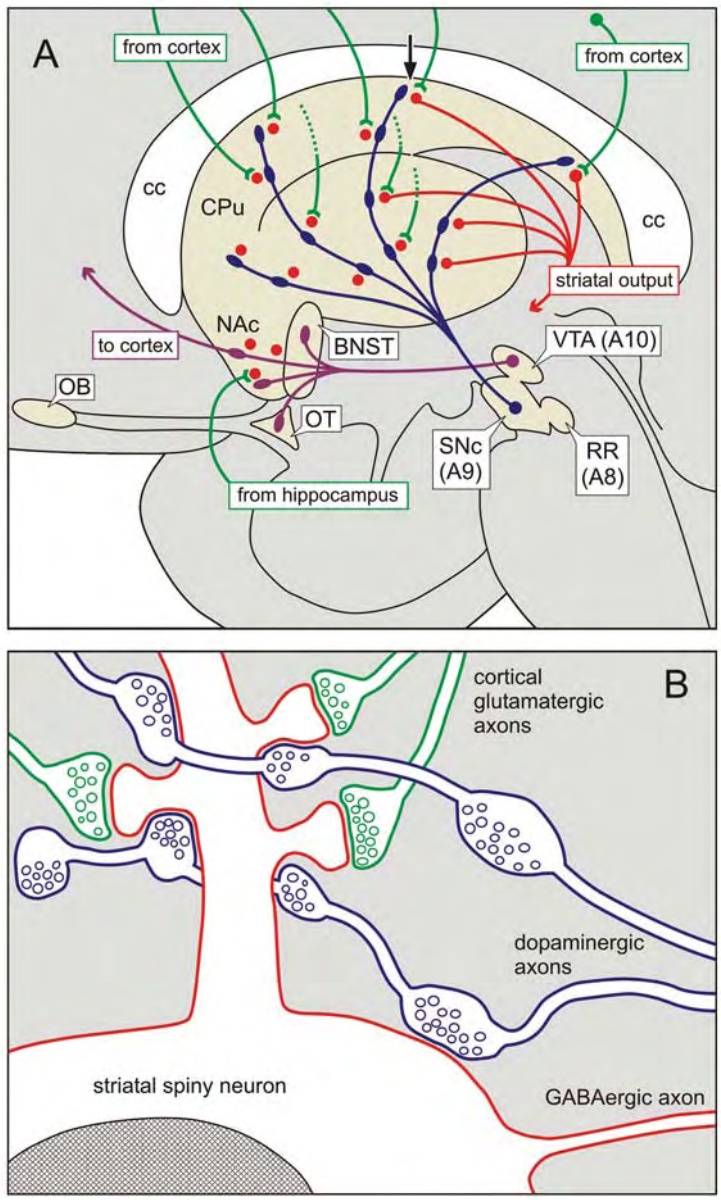

Fig. 18. Schematic representation of the organization of the mesostriatal and mesolimbic pathways (A), and of the synaptic organization of dopaminergic terminals contacting medium spiny neurons in the striatum (B). In the schematic representation shown in A, although, as explained in the text, mesostriatal and mesolimbic pathways take origin from a continuum of dopaminergic cells distributed in the ventral midbrain tegmentum (which in fact are not subdivided in the diagram), for the sake of clarity the nigrostriatal pathway (shown in blue) is indicated as originating from the A9 cell group and mesolimbic pathways (shown in purple) from the A10 cell group. The diagram in A depicts the long journey of dopaminergic nigrostriatal axons coursing throughout the length of the caudate-putamen (CPu), and whose varicosities establish multiple synaptic contacts with medium spiny neurons (shown in red). These striatal neurons give origin to the striatal output, and are contacted by corticostriatal axons (shown in green). The synaptic arrangements established by these inputs are illustrated in B. Features of organization similar to those of the nigrostriatal pathway are shown for the dopaminergic input to the ventral

studies of Fallon and Moore, 1978; Bentivoglio et al., 1979; Druga, 1989). In relation to this feature, Gerfen (2004) emphasizes that the delineation of subgroupings of the nigrostriatal cells may be somewhat arbitrary. Retrograde labeling after the injections of fluorescent tracers in the striatum combined with catecholamine histofluorescence in the rat revealed that only 5% or less of the nigrostriatal neurons are nondopaminergic (van der Kooy et al., 1981).

The organization of the nigrostriatal projections in the mouse, studied with lectin-conjugated HRP as tracer, was reported to be similar to that of the rat (Mattiace et al., 1989).

Both in the rat (Beckstead et al., 1979) and in the mouse (Mattiace et al., 1989) no strict topographical organization of the afferent projections from the ventral midbrain tegmentum was found in the rostrocaudal dimension of the striatum. This suggested that fibers efferent from each locus of the SN are distributed over most or all of the length of the striatum, although the actual length of individual nigrostriatal fibers turned out to be very difficult to verify.

The nigrostriatal projections are ipsilateral, but in studies performed with retrograde tracing a minor crossed contingent, arising from approximately 1% of SNc-VTA cells in the rat, has been identified (Fass and Butcher, 1981; Swanson, 1982; Altar et al., 1983). In double labeling experiments the contralateral mesostriatal pathway was found to contain catecholamines (thus strengthening its dopaminergic nature) and 50% of the cells of origin of this pathway contain the peptide CCK (Fallon et al., 1983).

Differences in the crossed projections have been reported between the rat and the mouse, since it has been suggested that in the latter species the VTA and the retrorubral field, but not the SNc, contribute sparse crossed projections to the striatum (Mattiace et al., 1989). In addition, inter-strain differences have been reported in mice; for example, crossed projections were documented in the CBA strain, but not in the BALB/c strain (Mattiace et al., 1989).

The reversal dorsoventral axis on the basis of which the ventral sheet of SNc and VTA cells project dorsally in the forebrain and the dorsal sheet project ventrally in the forebrain (Fig. 17), which led to the subdivision of the dopaminergic cells into dorsal and ventral tiers, has already been dealt with in Section 2.4.

In terms of the functional subdivisions determined in the striatum by corticostriatal projections, the motor striatum is innervated mainly by the lateral portion of the SNc, the associative striatum mainly by the medial portion of the SNc and VTA, and the limbic striatum mainly by VTA neurons extending into the medial SNc (Joel and Weiner, 2000).

Nigrostriatal axons are represented by relatively thin fibers, which exhibit a range of calibers: thin (0.1–0.4 μm) and smooth fibers, slightly thicker fibers (0.2–0.8 μm) with more frequent varicosities, and a minority of fibers of slightly larger calibre with large bulbous

←

striatum, reaching the nucleus accumbens (NAc), olfactory tubercle (OT), and distributed also to the bed nucleus of the stria terminalis (BNST). Medium spiny neurons of the NAc receive input from the hippocampus (shown in green). Other abbreviations: cc, corpus callosum; OB, olfactory bulb; RR, retrorubral area (cell group A8); SNc, substantia nigra, pars compacta (cell group A9); VTA, ventral tegmental area (cell group A10). The diagram in A illustrates the convergence of dopaminergic and cortical boutons on the same dendritic spines of striatal projection neurons, and the other features of synaptic arrangement which are explained in the text (see Section 5.2).

varicosities. In particular, relatively thin and smooth fibers with few varicosities represent the most common type of dopaminergic nigrostriatal axon. In terms of their termination into the striatal compartments, this type of fiber arises from the dorsal tier neurons of midbrain dopaminergic cells and terminates preferentially in the striatal matrix (Fig. 17). A second type of dopaminergic axon is represented by the thicker fibers with numerous varicosities, which arise from the ventral tier neurons and innervate selectively the striatal patches (Fig. 17).

With reference to the A8, A9 and A10 cell groups, the compartmental organization of their efferents to the striatum was examined in detail in the cat with anterograde tracing, TH immunohistochemistry and AchE histochemistry (Jimenez-Castellanos and Graybiel, 1987). Neurons of the A8 and A10 cell groups were reported to target the extrastriosomal matrix in the striatum (with dense projections of the A10 neurons to the ventral striatum), and the A9 neurons were instead found to target preferentially the striosomes.

As mentioned above, dopaminergic nigrostriatal fibers are considered to travel for long distances throughout the striatum, and they establish multiple synaptic contacts with striatal neurons (Fig. 18A). The occurrence of varicosities is of crucial importance since, as explained below, they are the sites of DA release (Fig. 18B).

With an anterograde tracing approach that resulted in cell filling, Gauthier et al. (1999) could visualize a limited number of individual nigrostriatal axons, which were seen to exhibit two main patterns of collateralization (see also Parent et al., 2000). The axons of the first type reached the striatum directly, emitting at the most, one thin collateral in the GP along their course, and they branched profusely within a rather restricted rostrocaudal sector of the striatum, breaking up into numerous, very thin and highly varicose terminal arborizations. The axons of the second type gave off instead collaterals to extrastriatal structures on their way, and arborized rather poorly within the striatum.

The ultrastructural organization of dopaminergic boutons in the striatum has been extensively investigated (see, inter alia, the reviews of Smith and Bolam, 1990; Sesack, 2003). Extrastriatal inputs, including the dopaminergic one, terminate mainly on the more distal part of the dendritic tree of medium spiny neurons, while intrinsic inputs terminate mainly on the proximal parts of the dendritic shaft and on the cell body. A small proportion of dopaminergic axons also contact the cell body of striatal projection neurons (Fig. 18B).

More than 90% of the dopaminergic terminals form symmetric synapses with spines and dendrites of striatal projection neurons. Dopaminergic afferents in the striatum converge with asymmetric boutons of cortical terminals on individual dendritic spines of medium spiny neurons (Fig. 18B). In rats, a high proportion (approximately 40%) of spines of striatonigral neurons receive convergent synaptic input from cortical terminals and dopaminergic terminals. This pattern of synaptic organization provides a strong indication that DA regulates in the striatum the flow of information transmitted by cortical synapses which establish contacts on the same dendritic spines.

The synaptology of dopaminergic axons in the striatum also exhibits other striking and complex features (Sesack, 2003). These axons release DA at symmetric synapses formed by varicosities (Fig. 18B), acting on the DA receptors within or closely adjacent to the synapse. Some of the synaptically released DA diffuses away from the synaptic cleft, exerting parasynaptic actions on DA receptors located at a distance from the synapse.

DA can exert its action on striatal cells via different mechanisms, which include the modulation of different voltage-dependent conductances and indirect effects on excitatory and inhibitory synaptic transmission (reviewed by Nicola et al., 2000). Because DA can diffuse away from its site of release, cellular responses to exogenous DA are likely to be identical or similar to those that follow the synaptic release of DA (Nicola et al., 2000).

DAT immunolabeling of axon terminals in the striatum revealed that DAT is concentrated near aggregates of synaptic vesicles, with less frequent labeling of intervaricose segments (Nirenberg et al., 1996b). These findings indicated that DAT is strategically located to facilitate DA uptake in nigrostriatal axons.

6. DOPAMINE MODULATION OF BASAL GANGLIA RELAYS

6.1. DOPAMINE MODULATION OF STRIATAL OUTPUT THROUGH THE DIRECT AND INDIRECT PATHWAYS

As mentioned in Section 4.3, the main output systems of the striatum are currently viewed as being organized into direct and indirect projection pathways originating from two populations of striatal GABAergic medium-sized spiny projection neurons (Fig. 16). In terms of dopaminergic modulation of these pathways, the direct pathway is dominated by D_1 receptors, expressed at a high level by striatonigral substance P-containing neurons. The indirect pathway is mainly regulated by D_2 receptors, expressed by striatopallidal neurons which contain enkephalin.

The segregation or abundance of D_1 and D_2 DA receptor subtypes on the direct and indirect striatal projection neurons indicates that the dopaminergic input arising from midbrain cell groups affects differentially the function of the striatal neuronal populations. At the striatal level, DA appears to facilitate the striatonigral neurons of the direct pathway through D_1 receptors, and to inhibit the striatopallidal neurons of the indirect pathway through D_2 receptors.

As discussed further in this chapter (see Sections 10.2 and 11.2; see also the reviews of Nicola et al., 2000; Smith and Kieval, 2000), both the anatomical segregation of the direct and indirect pathways and the segregation of the D_1 and D_2 receptors in subsets of striatal projection neurons giving origin to the two pathways is not exclusive. From the anatomical point of view, collaterals reaching both the GPi and the GPe from the same striatal neurons may subserve a crosstalk between the direct and indirect pathways. In addition, sensitive molecular biology techniques, such as RT-PCR, have shown colocalization of different receptors subtypes, though at low levels. In particular, striatonigral neurons also contain low levels of D_2 receptors and striatopallidal neurons, low levels of D_1 receptors.

Despite these concerns, several lines of evidence support the processing of neural information via separate striatal cell populations of the direct and indirect pathways. A confirmation of this parallel processing has derived also from an animal model, represented by conditional ablation of striatal neuronal types containing the DA D_2 receptor by using immunotoxin-mediated cell targeting in the mouse (Sano et al., 2003). In these mutant mice, the elimination of the majority of striatopallidal medium-sized spiny neurons and cholinergic interneurons expressing the D_2 receptors caused, besides molecular changes, hyperactivity of spontaneous movement and reduced motor activation

in response to DA stimulation. These findings support the role ascribed to the indirect pathway in the modulation of behavior.

6.2. DOPAMINERGIC INNERVATION OF THE GLOBUS PALLIDUS AND SUBTHALAMIC NUCLEUS

Observations made with histofluorescence had suggested that nigrostriatal fibers make along their way contacts *en passant* with neurons of the GP (Lindvall and Björklund, 1979). On the other hand, a dopaminergic fiber plexus was also seen in the STh (Björklund and Lindvall, 1984), and SNc projections to the STh were described with retrograde tracing (Campbell et al., 1985).

Therefore, inputs to both the GP and the STh, structures embedded within fiber bundles, are very difficult to investigate with retrograde tracing due to the problem of the potential tracer uptake by fibers passing through the injected area. Despite such methodological difficulties, refined techniques of anterograde tracing and/or retrograde tracer application have confirmed in the last years that the GP (both pallidal segments) and the STh receive projections from neurons of the SNc and VTA, whose dopaminergic nature was confirmed with TH immunohistochemistry (Hassani et al., 1997; Gauthier et al., 1999). Dopaminergic innervation of the STh from midbrain DA neurons was confirmed with the same strategy also in primates (François et al., 2000; see also Hamani et al., 2004).

The above-mentioned study of Gauthier et al. (1999), based on the tracing of single anterogradely labeled axons in the rat, has confirmed that the GP and STh are innervated by collaterals of the nigrostriatal bundle. In particular, these extrastriatal structures are innervated by the type of nigrostriatal axon which gives off thin and varicose collaterals along its way and does not display dense terminal fields in the striatum. The terminal domains of these fibers consist instead of a relatively limited number of thin and varicose fibers, scattered among neurons of both the pallidal segments and STh.

DA modulates in the STh neurons the activity of the glutamatergic cortical afferents and GABAergic pallidal afferents (Hamani et al., 2004). Dopaminergic terminals, which contact mainly the neck of dendritic spines, establish synaptic contacts with the distal dendrites of STh neurons. These receive synaptic input also from thalamic fibers deriving from the intralaminar nuclei, serotonergic input deriving from the dorsal raphe nucleus and glutamatergic input from the motor cortex. Inhibitory pallidal afferents innervate mostly the proximal dendrites and the cell body of STh neurons.

7. THE VENTRAL STRIATUM AND THE MESOLIMBIC SYSTEM

7.1. THE NUCLEUS ACCUMBENS

The NAc is the major component of the ventral striatum, and of the so-called limbic striatum. This striatal region corresponds to the entire anterior and ventromedial sector of the striatum and continues anteriorly into the NAc and the olfactory tubercle (Heimer and Wilson, 1975; Nauta, 1989). In the NAc, the principal neurons, which are the medium spiny projection neurons, make up approximately 90% of the total neuronal population and are generally similar to those of the striatal (i.e., neostriatal) counterpart. The local circuit neurons represent approximately 10% of the NAc neurons and vary greatly in size,

ranging from very small neurons to relatively large cholinergic interneurons (see for review Meredith, 1999).

A compartmental inhomogeneous organization of neuropeptides, DA and calbindin has been demonstrated in the NAc, but overall the chemoarchitecture and connectivity indicated that the NAc is not organized into patch and matrix compartments equivalent to those in the dorsal striatum, but is instead organized in several compartments with different chemoarchitectural features and different input-output relationships (Voorn et al., 1989).

Intense investigations on the cellular and molecular chemical neuroanatomy of the NAc, as well as on its connections, neuropharmacology and electrophysiology, have identified two main subterritories, namely the shell and the core (initially identified by Herkenham et al., 1984; Zaborsky, 1985); a third subdivision, represented by the rostral pole, has also been recognized (see for review Kelly, 1999; Meredith, 1999; Zham, 1999, 2000). The organization in subregions has led to theories on a modular function of the NAc as a complex of 'neuronal ensembles' (Pennartz et al., 1994). The shell and the core of the NAc have been demarcated also in primates, and calbindin is the most consistent marker for the shell across species (Haber, 1999).

Together with the olfactory tubercle, the NAc is characterized by its limbic input from the amygdala and allocortical regions (Heimer and Wilson, 1975; Nauta, 1989). Inputs to the NAc derive from the prefrontal cortex and limbic cortical fields, as well as hippocampal formation and amygdala (Meredith, 1999; Zham, 1999). Thalamic inputs to both the core and the shell of the NAc originate mainly in the midline nuclei, and derive in part also from the intralaminar nuclei. In particular, neurons projecting to the NAc are very dense in the thalamic paraventricular nucleus, a small structure which belongs to the midline group of thalamic nuclei and represents a key node in basal ganglia-limbic circuits (see Section 8.1). It has been reported in the rat that axons of the thalamic paraventricular nucleus which innervate the NAc give off also collaterals to prefrontal cortex (Bubser and Deutch, 1998). The NAc is also recipient of neuromodulatory input from hypothalamic (the preoptic area and the lateral hypothalamus) and brain stem structures.

The NAc core and the shell show differences in their input–output organization. For example, although hippocampal projections reach both the core and the shell, the ventral subiculum projects to the shell, whereas the dorsal subiculum projects to the core. Different regions of the prefrontal cortex also target differentially the shell and core: the prelimbic area projects to the core, whereas the infralimbic and piriform cortices project to the shell (Berendse et al., 1992). Amygdaloid fiber subsets are also differentially distributed to the two main NAc territories.

The core subregion of the NAc connects extensively to classic basal ganglia output structures, such as the ventral pallidum, STh and SN. In simple terms, it can be stated that the NAc core resembles the caudate-putamen, whereas the NAc shell region is more closely associated with the limbic system than the other regions of the ventral striatum. In particular, on the basis of the organization of the NAc subregions and their circuits, the NAc core has been associated with voluntary motor functions, whereas the shell has been rather associated with the 'extended amygdala'. Together with other regions of the CNS, the NAc shell is a key region initiating and maintaining the rewarding action of drugs of abuse (see, for example, McBride et al., 1999; Di Chiara, 2002) and therefore a key part of the brain reward system.

The NAc shell is a distinct region, not only due to its chemoarchitectural and pharmacological organization, but also on the basis of its preferential projections to

subcortical limbic regions, such as the lateral hypothalamus, ventromedial part of the subcommissural ventral pallidum and autonomic centers in the brain stem. The NAc shell also projects densely to the VTA. Through the ventromedial ventral pallidum, this basal ganglia output is conveyed to the thalamus, and especially to the medial portion of the thalamic mediodorsal nucleus (MD). The MD in the rat has strong reciprocal connections with the dorsal prelimbic and agranular insular cortex. In turn, these cortical fields project massively to the core of the NAc and adjacent parts of the caudate-putamen. It is therefore interesting to note that via thalamocortical and corticostriatal pathways, neural information can be shunted from the shell to the core of the NAc (Zahm, 1999).

7.2. MESOLIMBIC PATHWAYS AND THE VENTRAL STRIATOPALLIDAL SYSTEM

Through the pathways of the 'mesolimbic system' (a term introduced by Ungerstedt in 1971), the basal ganglia provide an interface with limbic brain regions. This interface has been repeatedly implicated in psychiatric diseases, such as schizophrenia and other affective disorders, as well as in reward and addiction.

As emphasized by Beckstead et al. (1979), a 'dualism' of the nigrostriatal pathway was proposed since the histofluorescence studies of Andén et al. (1966) and Ungerstedt (1971). According to these pioneering studies, the nigrostriatal dopaminergic system was subdivided into a 'nigrostriatal component proper' originating in the A9 cell group, and a mesolimbic system arising from the A10 cell group and projecting to the NAc and olfactory tubercle, i.e. the striatal regions which, as mentioned earlier, receive their major telencephalic input from the hippocampal formation and amygdala. Ungerstedt (1971) also noted additional dopaminergic fibers extending from the A10 cell group to the central nucleus of the amygdala and the bed nucleus of the stria terminalis (BNST). However, in their seminal paper on the efferents of SN, Beckstead et al. (1979) observed that the VTA projections involved 'the full length of the striatum' instead of being limited to the NAc and olfactory tubercle. Moreover, VTA projections were found to be very dense in the NAc, but were also seen to expand dorsally in the ventromedial half of the striatum, whereas the NAc and the olfactory tubercle appeared to receive only a few fibers from the SNc (Beckstead et al., 1979). These data indicated that the 'nigrostriatal system proper' and the 'mesolimbic system' were not segregated, but also suggested that neocortical and allocortical striatal inputs were conveyed through different streams and modulated mainly by different, though overlapping, dopaminergic cell groups of the midbrain.

As mentioned earlier in Section 2.4, subsequent tracing studies have confirmed that a dualism between the SNc and VTA based on their different targets is not supported by the organization of their efferents, demonstrating that the neurons of origin of different sets of telencephalic projections do not exhibit clear-cut boundaries in the ventral midbrain (Fig. 12). However, the classical subdivision between the SNc and the more 'limbic-related' VTA is still widely adopted on the basis of the prevalence of limbic and limbic-related targets of VTA efferents.

As the NAc, the olfactory tubercle and the BNST are innervated primarily by the A10 cell group and they receive afferent inputs originating also in the medial part of the SNc (Fallon and Moore, 1978; Beckstead et al., 1979) (Fig. 18). As dealt with in detail by Björklund and Lindvall (1984), the 'mesolimbic pathways' provide dopaminergic inputs also to the islands of Calleja, formed by clusters of neurons surrounded by a dense plexus of dopaminergic terminals, to the olfactory bulb and the anterior olfactory nuclei, to the

amygdala and to the septum. Dopaminergic innervation of the septum is localized to the lateral septal nucleus.

Double retrograde labeling of the VTA neurons from the frontal cortex, lateral septum, NAc, CPu and lateral habenula did not point out a highly divergent collateralization of VTA neurons (Albanese and Minciacchi, 1983). VTA neurons bifurcating to more than one target did not seem to exceed 10% of the total labeled population, and were relatively numerous when the injections were placed in the frontal cortex, lateral septum or lateral habenula (see also Section 8.2).

DA-containing axons which originate from all the midbrain dopaminergic cell groups (A8, A9 and A10) establish mainly symmetric synaptic contacts on the dendrites and cell bodies of projection neurons and interneurons in both the NAc shell and the core, with no obvious differences in the ultrastructural features or synaptic relations of the DA profiles in these different regions (Voorn et al., 1986; Meredith, 1999). The actions exerted by DA on the NAc medium spiny neurons are complex, resulting in inhibitory and facilitatory effects (Zahm, 2000). In the NAc, DA is likely to have effects on voltage-dependent conductances similar to those occurring in the dorsal striatum, but, in addition, DA in the NAc exerts effects on the excitatory and the inhibitory synaptic transmission independent of the modulation of ion channels (Nicola et al., 2000).

Despite the similarity in ultrastructural features of dopaminergic terminals throughout the NAc, the morphology of DA-containing axons and axon terminals in the core is distinct from those in the shell (Voorn et al., 1989). The NAc shell has a lower density of DAT-labeled axons than the core (Nirenberg et al., 1997b). Since low expression of DAT in axon terminals may contribute to enhanced extracellular DA, this feature could play a role in determining extracellular DA diffusion, and in regional sensitivity to substances transported by DAT, such as psychostimulants and neurotoxins.

The investigation of DAT immunolabeling at the ultrastructural level (Nirenberg et al., 1997b) visualized the DAT in varicose and intervaricose segments of axons in both the NAc core and the shell, with an organization similar to that detected in the dorsal striatum. Symmetric synapses were seen in both the two main NAc subregions, but DAT-immunoreactive processes only rarely formed synaptic junctions. DAT was not detected over synaptic densities and was instead mostly distributed on the extrasynaptic portions of the plasma membranes, near appositions with somata, dendrites, dendritic spines and astrocytes.

Recent ultrastructural data have demonstrated in the rat that DA can modulate thalamic inputs in the NAc shell (Pinto et al., 2003). This latter study documented some degree of convergence of terminals deriving from the thalamic paraventricular nucleus and dopaminergic axon terminals on the same target dendrites or dendritic spines. On these targets, thalamic terminals were found to establish asymmetric synapses, whereas dopaminergic terminals established symmetric synapses or appositional contacts. Interestingly, no similar features were observed in the prefrontal cortex (Pinto et al., 2003; see also Section 8.2).

The ventral striatum (NAc and olfactory tubercle) send efferents to the ventral pallidum, which represents a ventral extension of the GP lying below the anterior commissure in the forebrain. The ventral pallidum also receives a dopaminergic innervation from the VTA (Beckstead et al., 1979), and noradrenergic innervation from the locus coeruleus.

In its close association with the ventral striatum, the ventral pallidum constitutes the ventral striatopallidal system (Heimer and Wilson, 1975), a circuit integrated in a

feed-forward circuit similar to the neocortico-basal ganglia-thalamocortical loop (Fig. 18). Thus, while the main outflow of the GP is to the STh, SN and ventral tier of thalamic nuclei (Section 4), the efferent projections of the ventral pallidum involve not only the STh and the SN and VTA in the midbrain, but also various limbic and limbic-related structures (Heimer and Wilson, 1975; Nauta, 1989; Heimer et al., 1991): the amygdala, the medial frontal and cingulate cortex, the lateral habenular nucleus, the hypothalamus and regions of the midbrain tegmentum. The thalamic target of the ventral striatopallidal system is represented by medial and midline regions, and especially the MD nucleus, including also the thalamic paraventricular nucleus.

Two anatomically and neurochemically different subdivisions have been described within the ventral pallidum, namely a dorsolateral and a ventromedial compartment (Zham and Heimer, 1988; Zahm, 1989). Recent data based on the combined DAT and TH immunoreactivity at the light and electron microscopic levels have shown that the DAT-positive axonal profiles are denser in the lateral than in the medial compartment of the ventral pallidum, whereas the TH-labeled axons show a complementary distribution, with higher density in the medial than in the lateral compartment of the ventral pallidum (Mengual and Pickel, 2004). This organization supports a dualism of the DA action in the ventral striatopallidal pathways.

Drugs of abuse increase DA concentration in the mesolimbic system, and the NAc is a major site of action of psychostimulant drugs (Di Chiara and Imperato, 1988). DA plays a different role in the NAc subregions not only in behavior, but also in addiction (reviewed by Di Chiara, 2002). In particular, repetitive nondecremental stimulation of DA transmission by drugs in the NAc shell results in abnormal strengthening of stimulus-drug associations, so that the drug reward mechanisms acquire in the shell subregion powerful incentive properties, becoming resistant to extinction. Adaptive changes, whose significance is still uncertain, also occur in the NAc core, in which repeated drug exposure induces a sensitization of drug-induced stimulation of DA transmission.

It is noteworthy to emphasize, in this context, that the dopaminergic system which arises mainly from the VTA and reaches the NAc as well as other forebrain sites, including the dorsal striatum, provide the major substrate of reward and reinforcement for natural rewards (positive natural stimuli associated with survival, such as food). In addition, as mentioned, addictive drugs, such as amphetamine and cocaine increase the level of synaptic DA in the NAc acting on DAT. In particular, the NAc has been implicated in the response to the motivational significance of stimuli, and the dorsal striatum in the learning and execution of behavioral sequences that permit an efficient response to those cues (Hyman and Malenka, 2001; Wise 2002). Altogether these circuits (Fig. 18) provide the neural substrates of compulsive drug use and its persistence, which imply synaptic plasticity phenomena. Dorsal striatal circuits, involved in normal habit leaning, may be of particular importance in the shift from controlled drug use to compulsive drug abuse (Berke and Hyman, 2000).

7.2.1. Dopaminergic innervation of the habenula

As described in detail by Björklund and Lindvall (1984), the dopaminergic fibers form a dense plexus in the lateral habenular nucleus. This contingent of innervation is concentrated in the medial portion of the lateral habenula. Björklund and Lindvall (1984) pointed out that substantial evidence from lesion and tracing experiments had indicated that such DA innervation originates in the A10 cell group. In the retrograde

tracing study of Takada et al. (1990), neurons projecting to the lateral habenula were found medially in the VTA and in the midline structures belonging to the A10 cell group (the interfascicular and central linear nuclei). However, a subsequent study based on retrograde tracing combined with TH immunohistochemistry in the rat (Li et al., 1993) showed that dopaminergic cells projecting to the lateral habenular nucleus are located not only in A10, but also in other cell groups, including A9 and the diencephalic cell groups A14 and A15. Throughout these cell groups, more than 90% of the neurons projecting to the lateral habenular nucleus were found to be nondopaminergic, though the dopaminergic innervation of the medial portion of the lateral habenula was confirmed to be very dense (Li et al., 1993).

Interestingly, experimental chronic amphetamine and cocaine administration induces degeneration confined to the lateral habenula and its main output pathway, the fasciculus retroflexus (reviewed by Ellison, 1994). The habenula is the key relay in the descending dorsal diencephalic system, consisting of stria medullaris, habenula and fasciculus retroflexus, which links the limbic forebrain and the basal ganglia with lower diencephalic and mesencephalic centers (see for review Ellison, 1994). Dopaminergic dysfunction in this circuit could be implicated in psychiatric diseases including schizophrenia (Ellison, 1994).

7.3. THE CONCEPT OF EXTENDED AMYGDALA IN INFORMATION PROCESSING WITHIN DOPAMINERGIC CIRCUITS

The anatomofunctional concept of the 'extended amygdala' has been introduced to define transitional territories between the amygdala and the adjacent basal forebrain territories. These regions were previously 'identified with the somewhat forbidding designation' (Alheid, 2003) of substantia innominata (indicating in Latin a substance 'with no name'). As stated by Alheid (2003), this term 'while possessing a certain lyricism, obscures the fact that it encompasses several distinct regions that are related to nearby forebrain systems'. Although still disputed, the concept of the extended amygdala has gained consensus because it provides a systematic classification of a brain region difficult to define from the anatomical and functional points of view.

The structures included in the extended amygdala are located in the continuum of gray matter stretching rostromedially from the temporal lobe to the forebrain. This territory extends from the amygdala to the BNST. The anatomical and functional entities here have been identified as columns of neurons which accompany the stria terminalis, and are also found ventrally in the subpallidal zone (i.e. in the territory previously named as substantia innominata) (Alheid et al., 1995; Alheid, 2003; De Olmos et al., 2004). The rostral amygdala-related cell columns of the forebrain appear to form two major 'corridors', one related to the central nucleus of the amygdala and the other related to the medial nucleus of the amygdala. Because of the similarity between the central and the medial amygdaloid nuclei and their respective rostral counterparts based on a number of earlier anatomical studies, De Olmos et al. (1985) suggested that this continuum could be viewed as an extension of the amygdala. This was defined as 'extended amygdala' by Alheid and Heimer (1988), a term referring collectively to both the medial and central divisions of this region. Thus, the caudal part of the area previously included in the substantia innominata is occupied by extensions of portions of the amygdala into the forebrain, which are related to the medial and central amygdala, respectively. The rostral part of

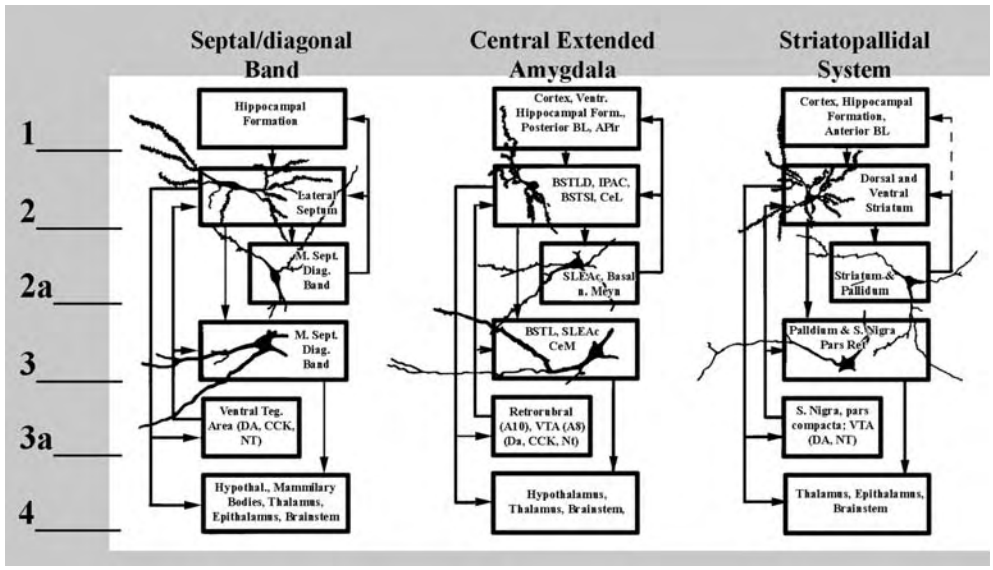

Fig. 19. Schematic representation of the structural plan for the basal forebrain, depicting three major cortico-subcortical 'corridors': the hippocampal-septal-diagonal band, the central extended amygdala; the cortico-striatal-pallidal pathway. In all these three systems, glutamatergic neurons (level 1) and cortical-like amygdala neurons project to GABAergic medium-sized spiny neurons (level 2). These cells project in turn to long slender projection neurons (level 3) in the diagonal band nuclei, in the central extended amygdala, but also to downstream targets such as the dopaminergic neurons of the ventral tegmental area. The long slender projection neurons provide a massive output (level 4) to the hypothalamus, thalamus and brain stem. Large interneurons with long slender dendrites (level 2a) receive input from medium spiny neurons and from thalamus, but rarely from the cortex. To varying degrees these provide feedback locally to medium spiny neurons, but also back to cortical areas from which the input to medium spiny neurons originated. Reproduced with permission from Alheid (2003).

the substantia innominata is instead represented by the ventral pallidum (Alheid et al., 1995; Alheid, 2003).

Both the medial and the central divisions of the extended amydala receive dopaminergic innervation from all the midbrain dopaminergic cells groups (A8, A9 and A10); the medial extended amygdala projects to the VTA, and the circuits efferent from the central extended amygdala also target the VTA, as well as the RRA (Alheid et al., 1995; De Olmos et al., 2004) (Fig. 19).

The study of the dopaminergic innervation of the principal components of the rat central extended amygdala by means of immunocytochemistry (Freedman and Cassell, 1994) indicated that dopaminergic fibers are most densely distributed in the dorsolateral subdivision of the BNST, and in the lateral and caudomedial portions of the central amygdaloid nucleus. These regions contain a relatively high number of GABAergic medium spiny neurons, supporting similarities with the organization of dopaminergic input to the striatum, and therefore supporting a common scheme of neural information processing which will be dealt with here.

The striatopallidal system and the extended amygdala have similar but also distinct and complementary chemical signatures (Alheid, 2003). On this basis, as well as on the basis of distinct circuitry, the extended amygdala is recognized as an entity independent from the

striatopallidal system, representing another pathway of information processing in which dopaminergic circuits are inserted (Fig. 19). The overall view of the extended amygdala as one of the main information channels in the cortical-subcortical basal forebrain systems (or cell 'corridors'), which also includes the hippocampal septal-diagonal band and the striatopallidal systems, proposes a common information processing scheme summarized in Fig. 19 (this figure depicts the central extended amygdala, but information processing in the medial extended amygdala 'corridor' proceeds through comparable steps). In this conceptual framework of a structural plan for the basal forebrain (Fig. 19), cortical neurons and cortical-like amygdala neurons direct their output to GABAergic medium-sized spiny neurons. In the septal-diagonal band circuits such medium spiny neurons are located in the lateral septum, in the central amygdala circuitry they are located in components which include the BNST, and in the striatopallidal system they reside in the striatum. Throughout these circuits, large interneurons with long slender dendrites receive inputs mainly from medium-sized spiny neurons. The 'second level' medium spiny neurons, in turn, project mainly to 'third level' projection neurons bearing relatively long and slender (relatively spine-poor) dendrites. To a lesser degree, the 'second level' medium spiny neurons also reach directly downstream targets, which include the VTA dopaminergic neurons. The 'third level' projection neurons provide massive output to the hypothalamus, thalamus and brain stem.

8. DOPAMINERGIC INNERVATION OF THE THALAMUS AND CEREBRAL CORTEX

8.1. DOPAMINERGIC INNERVATION OF THE THALAMUS

The dopaminergic fibers which innervate diencephalic structures will be dealt with here briefly, since most of the diencephalic DA-containing system is related to the dopaminergic cell groups of the hypothalamus, which are the subject of the chapter of Lookingland and Moore in this volume. The dopaminergic innervation of the STh was dealt with above in Section 6.2.

In the diencephalon, besides the hypothalamus, dopaminergic fibers have also been detected in the thalamic nuclei and in the habenula. It is interesting to note that in the thalamus dopaminergic fibers are distributed to nuclei that are also recipient of inputs from the ventral pallidum.

In particular, dopaminergic fibers are very abundant in the thalamic paraventricular nucleus. As mentioned above, this nucleus is a main relay in the basal ganglia circuits because it receives afferents from the ventral pallidum and gives origin to projections to the NAc. The thalamic paraventricular nucleus is composed of a mosaic of cell populations projecting also to the prefrontal cortex, hippocampus and amygdala (Bentivoglio et al., 1991). In neurons of the thalamic paraventricular nucleus which project to the NAc or to the amygdala, the nuclear phosphoprotein Fos, encoded by the immediate early gene c-*fos*, whose expression is a marker of neuronal functional activation, undergoes a circadian oscillation and is spontaneously induced during the activity period (Peng et al., 1995). The thalamic paraventricular nucleus is a site of interaction of NOS-containing and monoaminergic afferents derived from nuclei implicated in sensory gating and in the regulation of cortical activity (Otake and Ruggiero, 1995). Psychostimulants induce dose-dependently Fos expression in the

thalamic paraventricular nucleus, which has thus been involved in both the arousing properties and reinforcing aspects of these drugs (Deutch et al., 1998). The thalamic paraventricular nucleus is among the key structures in which motivational drives are represented in the brain (Sewards and Sewards, 2003).

Dense dopaminergic innervation of the thalamic paraventricular nucleus was detected since the initial studies based on histofluorescence (Lindvall and Björklund, 1974b) and TH immunoreactivity (Hökfelt et al., 1976; 1984a), and subsequently with anti-DA antibodies (Groenewegen, 1988). Groenewegen (1988) suggested that the dopaminergic innervation of the thalamic paraventricular nucleus could derive from DA-containing diencephalic cell groups, and this problem is still under investigation. With retrograde tract tracing it has been ascertained in the rat that the thalamic paraventricular nucleus receives fibers arising from the VTA, the RRA, and to a lesser degree from the A11 diencephalic dopaminergic cell group (Takada et al., 1990). However, in a subsequent investigation in which retrograde tracing was combined with immunohistochemistry (Otake and Ruggiero, 1995), it was reported that neurons projecting to the thalamic paraventricular nucleus from the A10 and A8 cell groups are nondopaminergic, and that dopaminergic inputs to these structures may derive instead from the diencephalic cell groups.

The periventricular DA system could also be responsible for the dopaminergic axons detected with DA immunohistochemistry in the rat lateral geniculate nucleus, and in particular in the ventral lateral geniculate nucleus (Papadopoulos and Parnavelas, 1990).

Projections to the thalamic MD from the SN and VTA have been repeatedly reported in several species (see, for the rat: Beckstead et al., 1979; Groenewegen, 1988), and seem to arise mainly from nondopaminergic cells, although SNc cells were proposed to account for dopaminergic innervation of the most medial portion of MD (Groenewegen, 1988). On the other hand, DA has been found to exert a marked modulation on the excitability of thalamic neurons recorded in slices of the rat MD and paraventricular thalamic nuclei (Lavin and Grace, 1998).

Interestingly, findings based on DAT and TH immunoreactivity in the monkey (Melchitzky and Lewis, 2001) indicate that the dopaminergic innervation is denser in the primate than in the rodent thalamus. In this study (Melchitzky and Lewis, 2001), dense dopaminergic axon and terminal elements were detected in the ventral and lateral portions of MD in the macaque monkey, as well as in the anteromedial nucleus and in the magnocellular division of the ventral anterior nucleus (which is the main target of the GABAergic nigrothalamic pathway in the monkey), and it was hypothesized that this innervation could derive from SNc dopaminergic cells.

8.2. DOPAMINERGIC INNERVATION OF THE CORTICAL MANTLE

The findings obtained by Dahlström and Fuxe in 1964 indicated the existence of a monoaminergic innervation of the cerebral cortex without a thalamic relay, but this idea seems initially heretic. However, methodological improvements of catecholamine histofluorescence with the sensitive glyoxylic acid histochemistry (Lindvall and Björklund, 1974a,b), together with the development of new techniques in experimental and chemical neuroanatomy mentioned earlier (see Section 1.1) convinced the neuroscience community that transmitter-characterized subcortical systems can innervate the cerebral cortex directly.

The dopaminergic innervation was the last to be identified in the cortex. By means of neurotoxic and electrolytic lesions of the noradrenergic system originating in the locus coeruleus, Thierry et al. (1974) could determine that DA was present in cortical fibers, and not in cortical cell bodies as believed initially (Thierry et al. 1973), and that the DA levels in the cortex were not affected by the lesions. In the same year, Hökfelt and coworkers (1974a,b), applying some sensitive modifications of the Falck-Hillarp technique, identified a plexus of dopaminergic fibers in the limbic cortex, with a patchy innervation of the entorhinal cortex. Interestingly, on the basis of these findings, Hökfelt et al. (1974b) hypothesized the potential involvement of dopaminergic limbic cortical innervation in major affective disorders such as schizophrenia, in line with current hypotheses based on pharmacological treatment. These pioneering observations paved way to another very stimulating and fertile field of research, related to the involvement of the central dopaminergic systems in psychiatric diseases. Thus, together with other brain systems, the dopaminergic circuits are still considered to play a key role in schizophrenia and its treatment (see for review Freedman, 2003).

Mesocortical projections are more loosely organized than the mesostriatal ones in terms of topography of their cells of origin (see for review Fallon and Loughlin, 1987). Projections to the prefrontal cortex arise mostly from the VTA, and those to the anterior cingulate cortex arise from cells distributed in the lateral portion of the VTA and in the medial portion of the SNc (Fig. 12). Cells projecting to the suprarhinal and perirhinal (Fig. 12) cortices reside throughout the SNc and VTA, with a lateral prevalence in the VTA, whereas those projecting to the entorhinal cortex are located in the VTA and in part, scattered in the retrorubral field. Dopaminergic axons branch within the cortex to reach more than one cortical area, and, as mentioned above, neurons located in the medial portion of the SNc were found to send collaterals to both the frontal cortex and subcortical targets, including the striatum and the septum (Albanese and Minciacchi, 1983).

Retrograde labeling from the frontal cortex combined with catecholamine histofluorescence in rat indicated that the vast majority (up to 90%) of VTA-frontal cells were catecholaminergic (Albanese and Bentivoglio, 1982), but different proportions were obtained in other studies (see Fallon and Loughlin, 1985).

DA-containing varicosities establish synaptic contacts preferentially on pyramidal neurons (Berger et al., 1991). In rodents, two main classes of cortical dopaminergic afferents have been defined (Berger et al., 1991). The first is preferentially distributed to the deep layers (V and VI) and exhibits a rostrocaudal gradient of decreasing density, from the prefrontal to the visual cortex. It is represented by smooth, sparsely varicose fibers, which originate mainly in the A10 cell group. The second type is distributed superficially to layers I-III and, although organized with a rostrocaudal gradient of density, is most dense in the anterior cingulate cortex. These fibers, which arise mainly from the A9 cell group, are thicker, more densely varicose and give off more collaterals than those of the first type. The neurons of origin of these two sets of dopaminergic cortical afferents have also a different peptidergic phenotype, since dopaminergic neurons projecting to the superficial layers do not express neurotensin, which is instead colocalized with DA in neurons which project to the prefrontal cortex and to the deep layers of other cortical targets.

The areal and laminar termination of dopaminergic fibers in the rat brain is illustrated in Fig. 20. In the prefrontal cortex, these fibers are distributed densely in layers V and VI, and scattered in the more superficial layers (Fig. 20A). In the most anterior (pregenual)

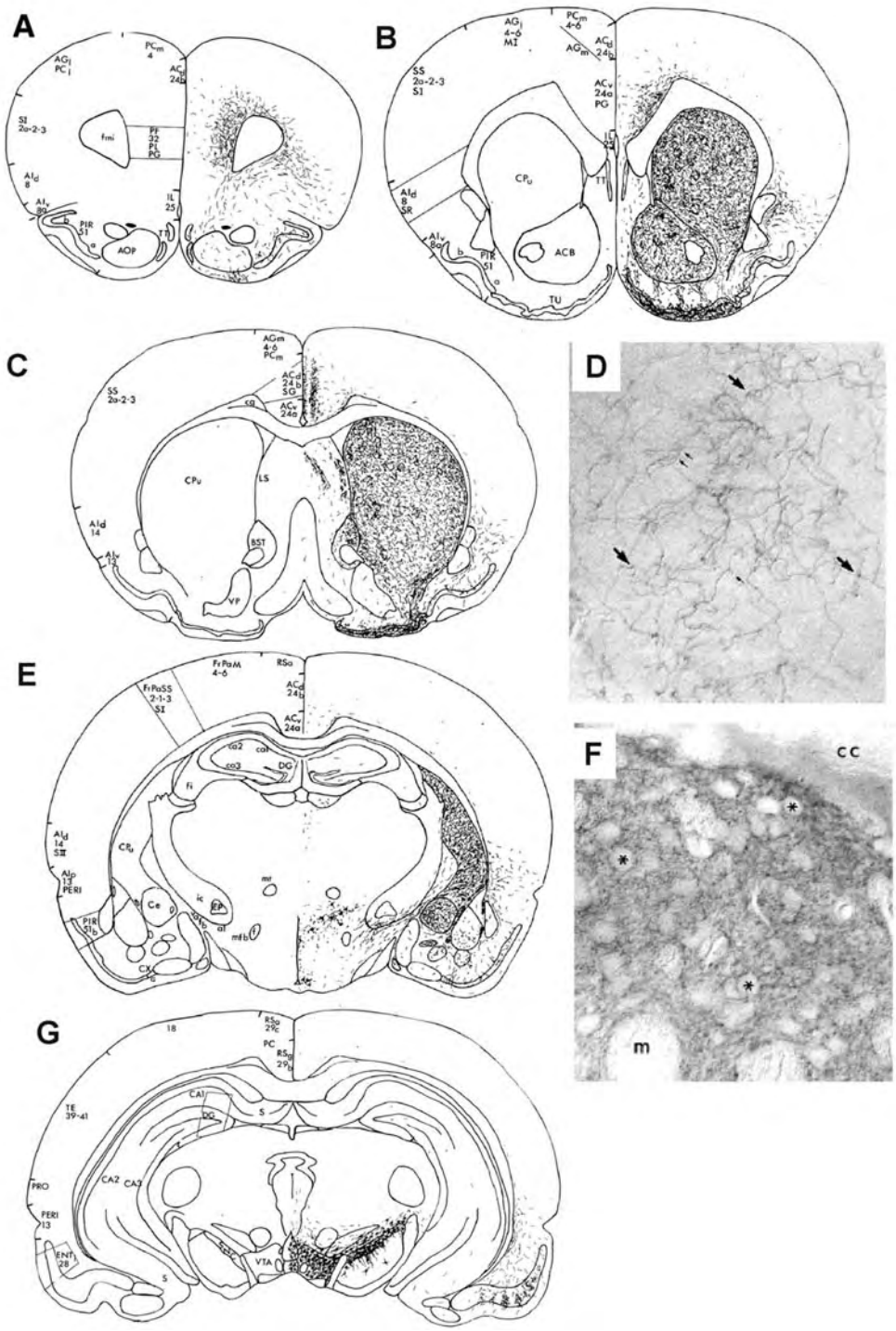

Fig. 20. Coronal sections through the rat brain (A–C,E,G), showing the pattern of dopaminergic innervation, to illustrate, in particular, the distribution of dopaminergic fibers in the cerebral cortex. Adapted from Fallon and Loughlin (1987). The images D and F, reproduced with permission from Sesack et al. (1998), illustrate the pattern of dopaminergic innervation, as visualized by immunoreactivity to the dopamine transporter. D: in layer III of

region of the anterior cingulate cortex, dopaminergic fibers are distributed in the deep cortical layers (Fig. 20B), whereas in the supragenual region the innervation is denser and more superficial, with fibers distributed in layers II/III and extending into layer I (Fig. 20C). Dopaminergic innervation is instead very sparse in the posterior cingulate retrosplenial cortex. The innervation of the perirhinal cortex does not show a preferential laminar distribution (Fig. 20E). In the piriform cortex dopaminergic, fibers are scarce and are found primarily in layers II/III (Fig. 20A,B,C,E). In the anterior portion of the lateral entorhinal cortex dopaminergic fibers have unique features, forming dense clusters in layers II/III (Fig. 20G) around islands of cells. The laminar distribution of cortical fibers in different cortical regions is summarized in Fig. 21.

The cortical dopaminergic innervation exhibits striking species differences, and is more expanded in primates than in rodents (see the reviews of Berger et al., 1991; Lewis and Sesack, 1997). For example, the motor, premotor and supplementary motor areas are densely innervated in primates but not in rodents. In addition, in primates dopaminergic terminals innervate densely layer I throughout the cortical mantle, whereas in rodents only the anterior cingulate cortex, and to a lesser extent the entorhinal cortex, receive a dense contingent of dopaminergic fibers in layers I–II.

The dopaminergic innervation of the prefrontal cortex has been repeatedly implicated in the modulation of normal cognitive processes, and in particular of working memory, as well as in cognitive dysfunction, such as age-related memory decline and alterations in neurodegenerative disorders including Parkinson's disease, as well as in psychiatric diseases.

The ultrastructural observations of Sesack et al. (1998) indicated that considerable extracellular diffusion of DA in the prelimbic prefrontal cortex may result, at least in part, from a paucity of DAT content in dopaminergic mesocortical axons, as well as from a distribution of DAT protein at a distance from synaptic release sites. In this study, DAT-immunoreactive profiles in the striatum and in the cingulate cortex included both varicose and intervaricose segments of axons, but intervaricose axon segments predominated in the prelimbic cortex.

In the rat prefrontal cortex, TH-immunoreactive terminals were seen to form occasionally symmetric synapses onto spines and dendrites, but were more commonly apposed to dendritic structures without establishing conventional synapses (Pinto et al.,

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the rostral portion of the anterior cingulate cortex immunoreactive fibers exhibit the branching (small arrows) and beading (large arrows) characteristic of termianl fibers. F: in the dorsolateral striatum, immunoreactivity is localized to the neuropil beneath the corpus callosum (cc); perikarya (asterisks) and bundles of myelinated axons (m) are unlabeled. Abbreviations: AC, anterior cingulate cortex; ACB, nucleus accumbens; AG, agranular cortex; AOP, anterior olfactory nucleus, posterior part, BST, bed nucleus of the stria terminalis; Ce, central amygdaloid nucleus; CPu, caudate-putamen; DG, dentate gyrus; ENT, entorhinal cortex; Ep, entopeduncular nucleus; f, fornix; Fi, fimbria of the hippocampus; fmi, forceps minor of the corpus callosum; Fr, frontal cortex; ic, internal capsule; IL, infralimbic cortex; LS, lateral septum; mt, mammillothalamic tract; mfb, medial forebrain bundle; PC, posterior cingulate cortex; PCm, paracentral thalamic nucleus, medial part, PCl, paracentral thalamic nucleus, lateral part; PIR, piriform cortex; RSa, retrosplenial agranular cortex; RSg, retrosplenial granular cortex; S, subiculum; SNC, substantia nigra, pars compacta; SS, somatosensory cortex; TE, temporal cortex; TU, olfactory tubercle; VP, ventral pallidum; VTA, ventral tegmental area.

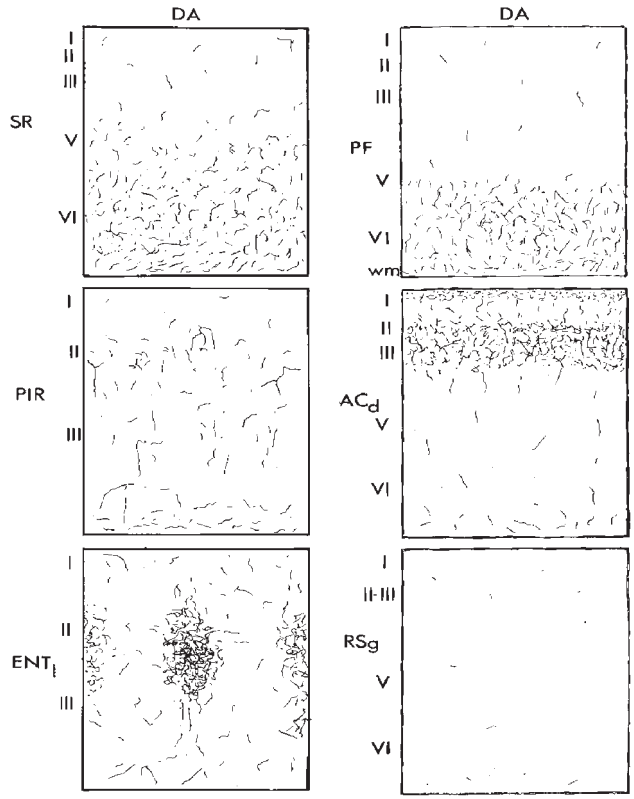

Fig. 21. Summary diagram of the laminar distribution of dopaminergic fibers in different fields of the rat cortex. Adapted from Fallon and Loughlin (1987). Abbreviations: ACd, dorsal anterior cingulate; ENTl, lateral entorhinal; PF, prefrontal; PIR, piriform; RSg, granular retrosplenial; SR, suprarhinal.

2003). At variance with the ultrastructural features observed in the NAc shell, dopaminergic axon terminals and boutons of thalamocortical axons deriving from the thalamic paraventricular nucleus were not found to converge on the same structures, such as dendritic shafts or spines (Pinto et al., 2003). Also the terminals of hippocampal fibers did not show obvious synaptic relationship with dopaminergic terminals in the prefrontal cortex (Carr and Sesack, 1996), indicating a segregation of different sets of cortical inputs.

An interesting study was performed on cortical slices (from young adult ferrets) with paired whole-cell recording to study the influence of DA on local inhibitory circuits (Gao et al., 2003). In this investigation, DA was found to depress inhibitory transmission between fast-spiking interneurons and pyramidal neurons, but enhanced inhibition between nonfast-spiking interneurons and pyramidal cells.

8.3. DOPAMINERGIC INNERVATION OF THE HIPPOCAMPUS AND OF THE SUBEPENDYMAL ZONE AND ROLE OF DOPAMINERGIC PROJECTIONS IN NEURAL PRECURSOR PROLIFERATION

A sparse dopaminergic innervation of the hippocampus has been described initially in the rat (Verney et al., 1985) and confirmed in primates in subsequent studies

(reviewed by Lewis and Sesack, 1997). In the rat, dopaminergic fibers were seen to target the subiculum and the ventral part of the Ammon's horn, whereas only sparse fibers were found in the dentate gyrus (Verney et al., 1985). Subsequent anterograde and retrograde tracing experiments in the rat (Gasbarri et al., 1994) reported that fibers reaching the hippocampus derive mainly from the VTA and also from the SNc. These dopaminergic fibers terminate in the ventral subiculum (stratum oriens and stratum moleculare), and in the adjacent CA1 field (strata oriens, pyramidale, suprapyramidale and moleculare), whereas in the dorsal hippocampus VTA fibers were found to be distributed in the CA1 polymorphic layer (Gasbarri et al., 1994).

Dopaminergic fibers identified with DAT immunoreactivity were recently identified in the mouse subgranular zone of the dentate gyrus, where stem cells are located in the adult brain, with only sparse immunoreactivity in the adjacent hilus or granule cell layer (Höglinger et al., 2004). In the subgranular zone, dopaminergic nerve terminals were found to contact proliferating cells (Höglinger et al., 2004), implicating DA in the modulation of adult neurogenesis.

It has been proposed that the dopaminergic innervation of the hippocampus could play a role in the selective retention of memory events before reward (Otmakhova and Lisman, 1998a). DA was found to exert a strong control of transmission in the perforant path, inhibiting the response to perforant path stimulation, but not the response of Schaffer collateral input (Otmakhova and Lisman, 1998b). Recent data in slices have reported that DA strongly depresses cholinergic gamma oscillations in the CA3 field of the rat hippocampus (Weiss et al., 2003).

As mentioned above, recent interesting findings pointed out a role of dopaminergic innervation in the control of neurogenesis in the adult forebrain. Besides the dopaminergic innervation of the hippocampal subgranular zone, dopaminergic fibers (probably deriving from the VTA) were also identified in the adult mouse subependymal zone, where they established anatomical and functional contacts with highly proliferative cell precursors (Höglinger et al., 2004). These features of dopaminergic innervation were found to be conserved in the human brain (Höglinger et al., 2004). The subependymal or subventricular zone of the anterior lateral ventricle, which is immediately adjacent to both the caudate nucleus and the NAc, represents another site of neurogenesis in the adult mammalian brain. The neurons generated in this region migrate along the so-called rostral migratory stream to the olfactory bulb where they differentiate into neurons (see references in Baker et al., 2004; Höglinger et al., 2004). In the subependymal zone, DA was found to control cell proliferation through D_2 receptors (Höglinger et al., 2004).

Destruction of dopaminergic neurons in the VTA and SNc with 6-OHDA and MPTP in adult rodents markedly reduced the number of proliferating cell precursors in the subventricular zone and in the dentate gyrus, providing strong indication for an implication of DA in the regulation of neurogenesis in the adult brain (Baker et al., 2004; Höglinger et al., 2004). Consistent with evidence that DA represents a stem cell regulator, mitotic activity was found to be reduced in the subependymal zone and the number of neural precursor cells markedly decreased in the olfactory bulb and dentate gyrus of individuals with Parkinson's disease; altogether these data are of special interest in view of the potential use of neural progenitor cells in brain repair processes (Höglinger et al., 2004).

9. DOPAMINE RECEPTORS: INTRODUCTORY REMARKS

9.1. GRADIENTS OF DENSITY

This part of the chapter will deal with the distribution of the DA receptors in the brain, in order to provide an overview of the sites of DA action. As in the previous sections, we will mainly refer to the rat brain, with a comparison with humans and nonhuman primates. Data on DA receptors in the human brain are also dealt with in the chapter of Hurd and Hall in this volume.

Since the concentration of receptors provides clues for the efficacy of DA action at given brain sites, the distribution of each receptor subtype will be presented according to an overall indicative criterion of high, intermediate and low density, based on the comparison between literature data. The definition of high, intermediate and low density and their illustration in the distributional maps refer to the relative density of each receptor subtype and are not indicative of a relationship in density across different receptor subtypes.

The wide distribution of DA receptors in the brain brings about the obvious consequences of the presence of more than one subtype of these receptors in the same brain sites. This does not necessarily imply, however, a colocalization of different DA receptors in single cells.

9.2. SUBTYPES OF DOPAMINE RECEPTORS

The physiological actions of DA are mediated by at least five different G protein-coupled receptor subtypes, which are classified into D_1-like family (D_1 and D_5) and D_2-like family (D_2, D_3 and D_4). The first identification of different DA receptor subtypes was made by Spano et al. (1978). Thereafter two receptor subtypes, named D_1 and D_2, were classified on the basis of their stimulatory or inhibitory activity on adenylyl cyclase (Kebabian and Calne, 1979).

After the introduction of gene cloning methodologies, three new receptor subtypes, D_3, D_4 and D_5, were characterized over the years. Characterization of complementary DNA for all five receptor subtypes showed that D_1 and D_5 receptors share high homology in their transmembrane sequences; similarly, the transmembrane sequences of D_2, D_3 and D_4 receptors are conserved in the three receptor species (Missale et al., 1998).

The functions of D_1 and D_2 receptors, and in part of D_3, have been characterized in behavioral and biochemical studies, whereas the lack of highly selective compounds for the analysis of D_4 and D_5 receptors has hampered a full clarification of their functions.

9.3. RECEPTOR DETECTION AND ITS PITFALLS

The main methods for receptor localization analysis are: (1) ligand binding to the receptor recognition site, (2) mRNA transcript detection, (3) immunohistochemical detection of the translated receptor protein with specific antibodies. Although these methods provide powerful (and somewhat complementary) tools, each of these approaches, as every other technique, has some limitations.

The distribution of the DA receptor subtypes was initially studied by analysis of ligand recognition site on receptor through the binding technique, utilizing isotope-labeled ligands and autoradiography. This technique provides a direct assessment of the pharmacologically active receptor. The major drawback of this methodological approach consists in the limited availability of drugs highly selective for each of D_3, D_4 and D_5 receptors.

Detection of mRNA of each cloned DA receptor by means of in situ hybridization is a sensitive and specific methodology. mRNA, however, is almost exclusively contained in the neuronal cell body and, therefore, this technique does not allow the identification of receptors within dendritic arborizations or axon terminals. In addition, the presence of RNA transcript does not allow to determine to what extent the mRNA is translated into protein, and does not always correlate with the presence of active receptor protein or ligand binding sites.

Utilization of selective antibodies is the most recent tool for receptor visualization, based on immunohistochemistry. This experimental approach has the advantage of allowing the identification of the receptor protein throughout the neuron, which can also be achieved at the ultrastructural level, with high selectivity and sensitivity. However, as for the mRNA, the antigenic sites detected by the antibodies may also be present on a receptor protein not physiologically active in the process of synthesis, catabolism or transport.

The above limitations present in each of these techniques may account for the mismatches observed in the description of DA receptor localization provided by the various studies which are reviewed in this chapter.

10. D_1 RECEPTORS

10.1. OVERVIEW OF D_1 RECEPTORS

Since the initial classification of DA receptor into D_1 and D_2 subtypes, more than one decade has elapsed before the D_1 receptor was first cloned (Dearry et al., 1990; Monsma et al., 1990, Zhou et al., 1990). Differently from the D_2 receptor, the D_1 receptor gene lacks intronic sequences. Only one additional member of the D_1 receptor family has been cloned, termed D_5 for humans and D_{1B} for rodents (Grandy et al., 1991; Sunahara et al., 1991; Tiberi et al., 1991).

Like the D_2 receptor (see further, Section 11.1), the D_1 receptor belongs to the family of G protein-coupled receptors and is characterized by its stimulatory activity on adenylyl cyclase being coupled to either G_O or G_S protein (Spano et al., 1978; Kebabian and Calne, 1979; Herve et al., 1993). Biochemical and electrophysiological studies have also evidenced a D_1-like receptor coupled to stimulation of phosphoinositide turnover (Missale et al., 1998; Niznik et al., 2003).

The D_1 receptor subserves numerous psychomotor functions and, in concert with the D_2 receptor, produces the great majority of DA-dependent behaviors. In line with such multiple functions, D_1 receptors have a widespread distribution in the brain.

In all brain structures except the VTA, and with the exception also of the pituitary gland, D_1 receptors are present in higher density than D_2 receptors (Boyson et al., 1986).

The presence and density of D_1 receptors have been investigated in several studies (Aiso et al., 1987; Dawson et al., 1988; Richfield et al., 1989; Huang et al., 1992, Levey et al., 1993; Yung et al., 1995). Figure 22 depicts the relative density of dopamine D_1 receptor binding sites at selected representative levels of the rat brain in structures with high and medium concentration of this receptor.

The highest concentration of the D_1 receptor binding sites was found in the olfactory tubercle, NAc, CPu, SNr, and in the EP, the rodent homologous of the GPi (see Section 4). Medium concentration of D_1 receptor binding sites was found in several cortical fields and subcortical structures: the prefrontal, cingulate, parietal, piriform and entorhinal cortices, as well as in the olfactory bulb, major island of Calleja, claustrum, lateral septal nuclei, ventral pallidum, amygdala, hippocampus, substantia innominata, STh, throughout the thalamus, and in selected hypothalamic areas. In addition, medium concentration of D_1 receptor binding sites was found in the molecular layer of the cerebellar cortex and the retina. Medium/low amount of D_1 receptors was detected in the VTA and in the GP (i.e. the rat homologous of the GPe).

D_1 receptor mRNA is most abundant in the olfactory tubercle, NAc and CPu. Cells expressing D_1 receptor mRNA were also found in the neocortex, lateral septal nuclei, amygdala, hypothalamus (and in particular in the suprachiasmatic nucleus), in the thalamus and in the retina. D_1 receptor mRNA was instead absent in the VTA, GP, SNr, SNc and EP (Mansour et al., 1990, 1992; Fremeau et al., 1991; Le Moine et al., 1991; Meador-Woodroff et al., 1991b; Mengod et al., 1991; Weiner et al., 1991; Gaspar et al., 1995).

The lack of correspondence between receptor protein and mRNA in some areas is likely to be due to the transport of the receptor from the site of synthesis into terminal areas. In the cerebellar cortex, for example, D_1 receptors synthesized in the granule cell layer are transported to the molecular layer (Mansour et al., 1992).

10.2. D_1 RECEPTOR DISTRIBUTION IN THE RAT BASAL GANGLIA

Activation of D_1 receptors has little effect on locomotor behavior; however, D_1 receptors play a fundamental role in the control of movement in concert with D_2 receptors, as shown by studies on the synergism between these two receptor subtypes and on sensitization of D_1 receptors (Morelli et al., 1993a,b,c; Niznik et al., 2003).

In the CPu, D_1 receptors are mostly localized in medium-sized GABAergic neurons, whereas large aspiny cholinergic interneurons do not express either D_1 receptor protein or D_1 mRNA (Weiner et al., 1991; Huang et al., 1992; Bergson et al.,1995b).

Most of the striatal neurons containing D_1 mRNA, contain the peptides substance P and dynorphin; on the other hand, neurons which contain preproenkephalin A do not contain D_1 mRNA, showing a selective association of D_1 receptor with the direct pathway of striatal output (Le Moine et al., 1991; Yung et al., 1995) (see Section 6.1). Although located in different striatal neuronal populations (see, Section 11.2), the D_1 receptor acts in concert with the D_2 receptor to produce DA-mediated behaviors.

Ipsilateral cortical afferents to the striatum establish synaptic contacts with D_1 receptor more than D_2 receptor-positive spines (Hersch et al., 1995).

Ultrastructural studies in the striatum showed that D_1 receptor is present on dendrites and spines (heads and necks) postsynaptic to asymmetrical synapses and on postsynaptic densities on small synapses characteristic of DA terminals. The D_1 receptor is also

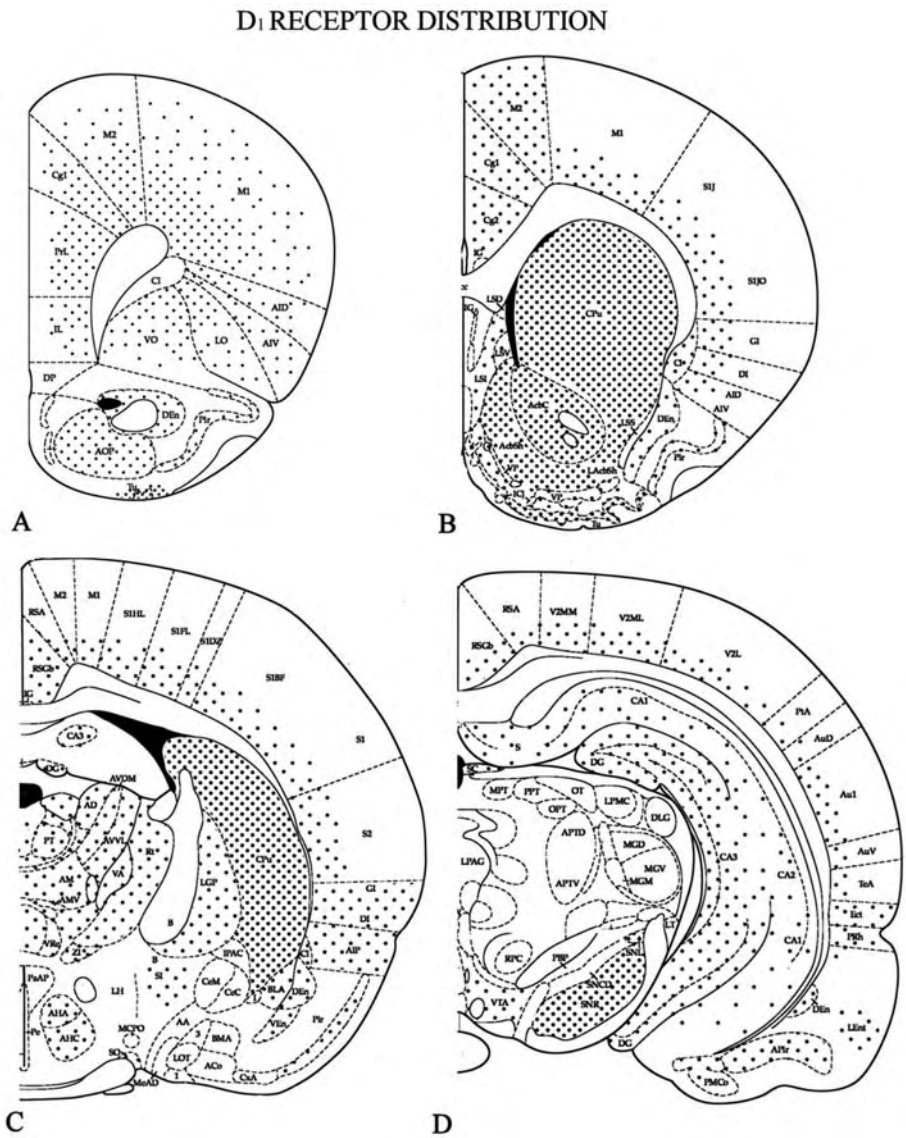

Fig. 22. Distribution of D_1 receptor in selected representative sections through the rat brain. The sections, taken from the atlas of Paxinos and Watson (1998), correspond to the following levels from bregma: A, + 3.20; B, + 1.60; C, − 1.60; D, − 5.30. In this and the following distributional maps (Figs. 25, 26, 28, 29), the dots illustrate the high and medium relative density of each receptor subtype, whereas the low density is not illustrated. Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; AD, anterodorsal thalamic nucleus; AVVL, anterior thalamic nucleus; AI, agranular insular cortex; AM, anteromedial thalamic nucleus; APT, anterior pretectal nucleus; BLA, basolateral amygdaloid nucleus; Cg, cingulate cortex; Cpu, caudate-putamen; Ce, central amygdaloid nucleus; Den, dorsal endopiriform nucleus; DG, dentate gyrus; Icj, islands of Calleja; IL, infralimbic cortex (frontal cortex); GP, globus pallidus; LO, lateral orbital cortex; LS, lateral septal nucleus; M, motor cortex; MG, medial geniculate nucleus; MM, medial mammillary nucleus; PBP, parabrachial nucleus; Pir, piriform cortex; PrL, prelimbic cortex (frontal cortex); S, subiculum; S1J, parietal cortex; SC, superior colliculus; SI, substantia innominata; SNc, substantia nigra compacta; SNl, substantia nigra lateralis; SNr, substantia nigra reticulata; SO, supraoptic nucleus; Tu, olfactory tubercle; VA, ventral anterior thalamic nucleus; VO, ventral orbital cortex; VP, ventral pallidum; ZI, zona incerta.

presynaptically located, however, in contrast to D_2 receptor, axon terminals containing D_1 receptor are rare (Huang et al., 1992; Levey et al., 1993; Hersch et al., 1995). In spite of the association of D_1 receptor with synaptic inputs, a high proportion of D_1 receptors are located extrasynaptically.

In the SN, the D_1 receptor concentration is highest in the SNr, relatively high in the pars lateralis and lower in the SNc (Altar and Hauser, 1987; Dawson et al., 1988). 6-Hydroxydopamine lesion of DA nigrostriatal neurons or ibotenic acid lesion of intrinsic SNr neurons do not modify D_1 receptor binding, indicating that D_1 receptors are presynaptically located on nerve terminals originating in the CPu (Savasta et al., 1986; Filloux et al., 1987b; Morelli et al., 1988). In line with these findings, degeneration of striatal intrinsic and projection neurons produced an almost total depletion of D_1 receptors in the CPu and strongly decreased D_1 receptors in the SNr; moreover, D_1 mRNA was not detected in the SNr (Fremeau et al., 1991; Le Moine et al., 1991). In addition, analysis of receptor localization with antibodies against D_1 receptors showed that D_1 receptor immunoreactivity was localized in axon terminals forming symmetrical synapses (Levey et al., 1993).

A topographical organization of projections from the CPu to the SN was observed, since neurons containing D_1 receptors and having rostral, central and caudal origin in the CPu correspond to medial, central and lateral terminations in the SNr, respectively (Altar and Hauser, 1987; Harrison et al., 1990). D_1 receptor immunoreactivity in both the striatum and the SN is illustrated in in Fig. 23.

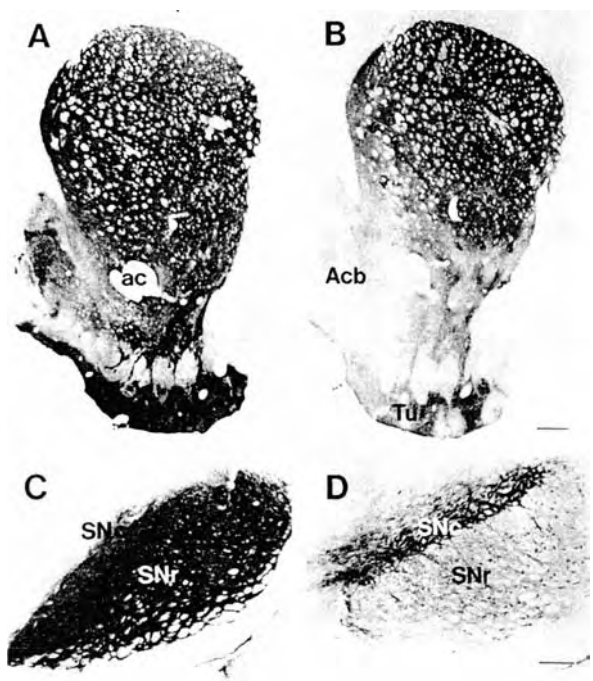

Fig. 23. Comparison of D_1 (A and C) and D_2 (B and D) immunoreactivity in the rat striatum, nucleus accumbens (Acb), olfactory tubercle (Tu) and substantia nigra (SN) compacta (c) and reticulata (r). Bars: A and B, 250 μm; C and D 200 μm. Reproduced with permission from Levey at al. (1993).

10.3. D_1 RECEPTOR DISTRIBUTION IN THE RAT CEREBRAL CORTEX

DA is involved in complex physiological and pathological conditions related to cognitive functions (see the chapter of Robbins in this volume). Therefore, the detailed localization of DA receptors in the cerebral cortex, and in particular in the prefrontal cortex, has been the subject of several studies aimed at the understanding of the role of each DA receptor subtype in functions such as working memory, or in neuropsychiatric disorders. These investigations reported the presence of D_1 receptor in the neocortex, mainly in neurons of prefrontal and cingulate fields. The D_1 receptor was also found at high level in the piriform, entorhinal and retrosplenial cortices. The D_1 receptor has both presynaptic and postsynaptic localization, with the postsynaptic more frequently observed (Huang et al., 1992; Levey et al., 1993).

In the prefrontal cortex, D_1 receptors were found to be present in layers II–VI, with highest density in layer V and VI; in layer VI cells containing D_1 receptors belong to nonpyramidal neurons. Since the D_2 receptor was mainly found in either large or small pyramidal cells, altogether the findings suggest that the two receptors are mainly found in different cortical cell populations (Vincent et al., 1993).

D_1 mRNA, similar to the D_1 receptor, has higher expression than D_2 mRNA. In the prefrontal cortex, D_1 mRNA was found to be most abundant in layer VIb. mRNA was also detected in layers VIa and V of the frontal cortex, and in layer II of the medial prefrontal, cingulate and insular fields (Weiner et al., 1991; Gaspar et al., 1995). In the frontal cortex, D_1 mRNA, similarly to D_2 mRNA, is expressed in GABAergic interneurons containing parvalbumin, whereas only 10% of the calbindin-positive neurons express D_1 mRNA. Double labeling showed that D_1 mRNA is also present in projection neuronal populations, including corticocortical, corticothalamic and corticostriatal neurons (Gaspar et al., 1995; Le Moine and Gaspar, 1998).

Figure 24 illustrates the laminar distribution of D_1 receptor immunoreactivity in different cortical areas.

10.4. D_1 RECEPTOR DISTRIBUTION IN THE RAT LIMBIC SYSTEM

The role of DA receptors in functions subserved by the limbic system, first of all reward and affective behavior, has been highlighted by behavioral studies which have pointed out a permissive role of the D_1 receptor in these functions (Sutton and Beninger, 1999).

In the NAc shell and in the olfactory tubercle, a fraction of D_1 receptors are expressed in cell patches surrounded by a high density of DA terminals, suggesting that in these compartments DA transmission mainly acts on D_1 receptors. However, the overall density of TH correlates with the density of both D_1 and D_2 receptors (Jansson et al., 1999). In the NAc core and shell, D_1 mRNA is coexpressed with D_3 mRNA in a subpopulation of neurons containing substance P. A significant proportion of NAc neurons, however, express only D_1 mRNA (Le Moine and Bloch, 1996). In the olfactory bulb, D_1 receptor is restricted to the internal granular and plexiform layers (Levey et al., 1993).

As for the amygdala, D_1 receptors are highly concentrated in the intercalated cells, with intermediate density of D_1 receptors in the magnocellular and parvicellular basolateral nucleus, whereas a low density is found in the central nucleus (Scibilia et al., 1992).

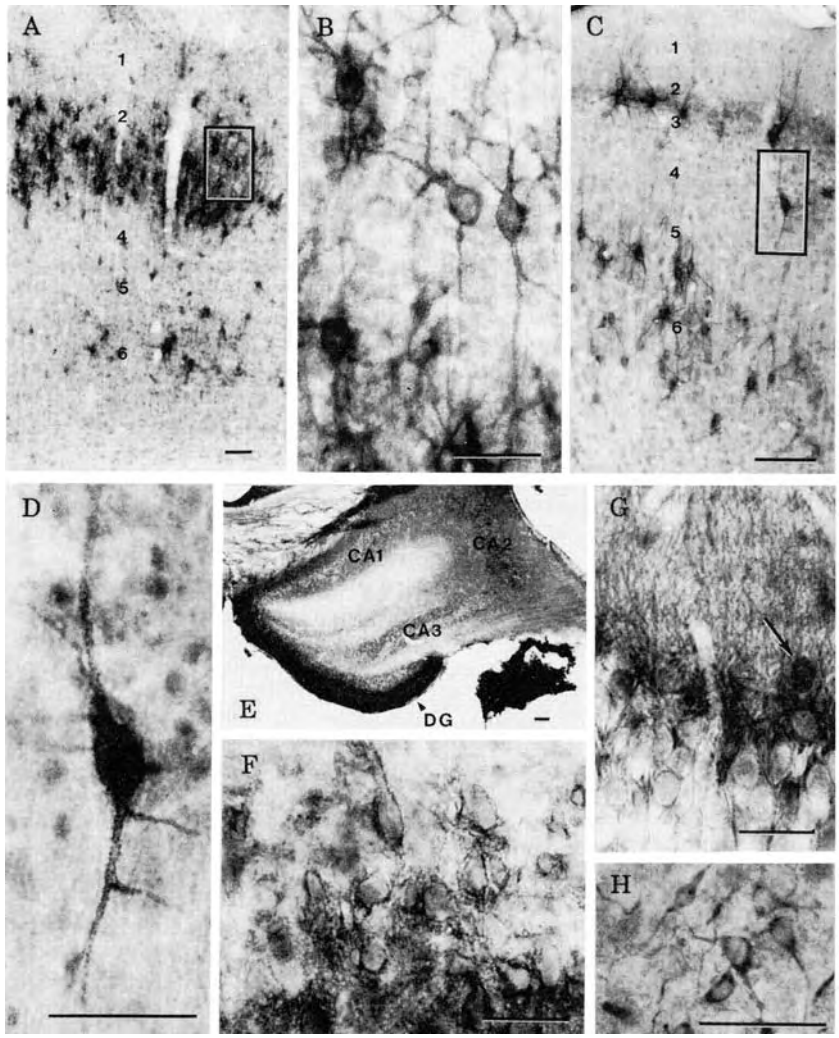

Fig. 24. D_1 receptor immunoreactivity in parietal cortex (A and B), cingulate cortex (C and D), hippocampal formation (E–G) and medial dorsal nucleus of the thalamus (H). In A and C, the numbers identify cortical layers and boxed areas are shown in B and D, respectively. Arrow in G identifies labeled dentate granule cells. Bars = 100 μm (A, C and E) or 50 μm (B, D and F–H). Reproduced with permission from Huang et al. (1992).

In the hippocampal formation, D_1 receptor binding is the highest in the molecular layer of the dentate gyrus and in the stratum moleculare. DA receptors in the hippocampal formation have a different laminar distribution: layers with high density of one receptor subtype exhibit low density of the other subtypes. In the entorhinal cortex, which contains the highest density of DA receptors, high D_1 receptor density is found in layers II, IV, V and VI. Moreover, the parasubiculum and Ammon's horn (stratum lacunosum moleculare) have high D_1 receptor density, whereas a few D_1 receptors are found in the presubiculum (Kohler et al., 1991).

D_1 mRNA is differentially expressed in distinct hippocampal regions: granule cells of the dentate gyrus express D_1 mRNA in dorsal but not in ventral regions; in contrast, the subicular complex is labeled in ventral rather than the dorsal regions (Fremeau et al., 1991). D_1 mRNA is localized in granule cells of dentate gyrus, whereas no D_1 receptor mRNA is detected in the hippocampal formation. It is therefore suggested that either D_1 receptor is transported within the dentate gyrus, or synthesized in extrahippocampal areas and then transported to the stratum molecolare of the hippocampal formation (Mansour et al., 1992). Figure 24 shows the compartmentalization of the D_1 receptor protein immunoreactivity, in the hippocampus.

In general, D_1 receptor is poorly expressed in the hippocampal formation as compared to the D_{1B}/D_5 receptor (see Section 14.4). It is therefore likely that the D_5 receptor is more involved in memory and learning processes than the D_1 receptor.

10.5. D_1 RECEPTOR DISTRIBUTION IN THE HUMAN AND NONHUMAN PRIMATE BRAIN

In humans and monkeys, D_1 receptors have a distribution similar to that described above in the rat brain, with the highest concentration in the striatum (caudate nucleus, putamen, NAc), olfactory bulb and SN. A good correlation between localization of D_1 mRNA in the rat and primate brains has also been described, although specific differences have been reported within each region (Mengod et al., 1991; Levey et al., 1993; Bergson et al., 1995b; Choi et al., 1995; Meador-Woodruff et al., 1996; Hurd et al., 2001).

D_1 receptor binding sites were found to be 10–20 times higher than those of D_2 receptor throughout primate neocortex. However, similar to the D_2 receptor, the D_1 receptor binding sites were found to be distributed according to a rostrocaudal gradient, with the highest concentration in the prefrontal cortex and the lowest in the occipital cortex (Lidow et al., 1991). D_1 receptor was detected in pyramidal neurons and concentrated in the dendritic spines of these cells, suggesting a primary role of D_1 receptor in the modulation of glutamate input to cortical pyramidal cells (Smiley et al., 1994; Bergson et al., 1995b).

A bilaminar pattern of the D_1 receptor binding was described in the human and monkey neocortex, with the highest labeling in the supragranular layers I, II and IIIa and the infragranular layers V and VI (Lidow et al., 1991; Huntley et al., 1992). The differential laminar distribution of D_1 receptors as compared to the D_2 receptors suggests that the two receptor subtypes subserve different functions in the cerebral cortex.

Among DA receptors, the D_1 receptor has been shown to play the most crucial role in cognitive functions. In the prefrontal cortex, the D_1 receptor is present in interneurons expressing parvalbumin (Muly et al., 1998). At the ultrastructural level, the D_1 receptor in the prefrontal cortex is associated with the Golgi apparatus and endoplasmic reticulum in the neuronal soma, with membrane vesicles in the proximal dendrites and with plasma membranes on distal dendrites located near asymmetrical synapses (Bergson et al., 1995b; Muly et al., 1998). The D_1 receptor is also associated with presynaptic axon terminals forming symmetrical synapses with dendritic shaft and soma (Muly et al., 1998). The majority of D_1 receptors in dendritic spines are connected to synapses which do not exhibit the features of dopaminergic elements or TH

immunoreactivity, suggesting that D_1 receptors are located at extrasynaptic sites (Smiley et al., 1994).

In the caudate nucleus, D_1 receptors are concentrated in the dendritic spines and shaft of projection neurons (Bergson et al., 1995b). Furthermore, while in the caudate nucleus D_1 mRNA is concentrated in calbindin-poor striosomes, in the putamen D_1 mRNA is more homogeneously distributed (Rappaport et al., 1993).

D_1 receptor mRNA is prominently expressed in the SNr neuropil, at variance with the rat brain, in which, as mentioned above, D_1 mRNA is absent in the SNc and SNr, as well as in the VTA, and in the external and internal segments of the GP (Mengod et al., 1991).

11. D_2 RECEPTORS

11.1. OVERVIEW OF D_2 RECEPTORS

The D_2 receptor was the first characterized receptor of the D_2-like subfamily of DA receptors and the first cloned DA receptor (Bunzow et al., 1988). Two isoforms of the D_2 receptor, namely short and long isoforms, are generated by alternative splicing of the same gene (Giros et al., 1989; Montmayeur et al., 1991). The D_2 short, which is highly expressed on dopaminergic cell bodies and axons, is prevalent in the mesencephalon and hypothalamus, whereas the D_2 long, which is prominently expressed at postsynaptic level, prevails in the CPu and NAc (Khan et al., 1998; Tan et al., 2002).

The D_2 receptor is coupled to G protein and has been mainly characterized as an inhibitor of adenylyl cyclase. The D_2 receptor also activates K^+ channels, stimulates phospholipase A_2 and affects Ca^{2+} channels.

D_2 receptors have been extensively investigated in the rat brain (Palacios et al., 1981; Jastrow et al., 1984; Boyson et al., 1986; Dawson et al., 1986; Bouthenet et al., 1987; Charuchinda et al., 1987; Richfield et al., 1989; Mansour et al., 1990; Ariano et al., 1993; Levant et al., 1993; Levey et al., 1993). Figure 25 depicts the relative density of D_2 receptor at selected representative levels of the rat brain.

The highest density of D_2 receptor, as measured by selective radiolabeled drugs or antibodies, was found in the NAc, olfactory tubercle, olfactory bulb (glomerular layer) and CPu. In the pituitary gland, D_2 receptors were found to be present at a high level in the intermediate lobe.

Medium-high density of D_2 receptors was found in the islands of Calleja, ventral pallidum, zona incerta, GP, central amygdala, in some cells of the anterior lobe of the pituitary, and at several sites in the forebrain: the laterodorsal septal area, hippocampus, subiculum, lateral habenula, STh, lateral mammillary bodies. D_2 receptors were also found with a medium-high density in various cortical fields: prefrontal, anterior cingulate, entorhinal and perirhinal cortices. In the brain stem, medium-high density of D_2 receptors was found in the VTA, SNc, ventral SNr, parabrachial nucleus, superior and inferior colliculi, dorsal raphe nucleus and locus coeruleus.

A medium-low density of D_2 receptors was detected in the sensorymotor, visual, piriform and retrosplenial cortices, in the bed nucleus of the stria terminalis, basolateral amygdala, thalamus, EP, SNr, anterior hypothalamic area and cerebellar lobules 9 and 10.

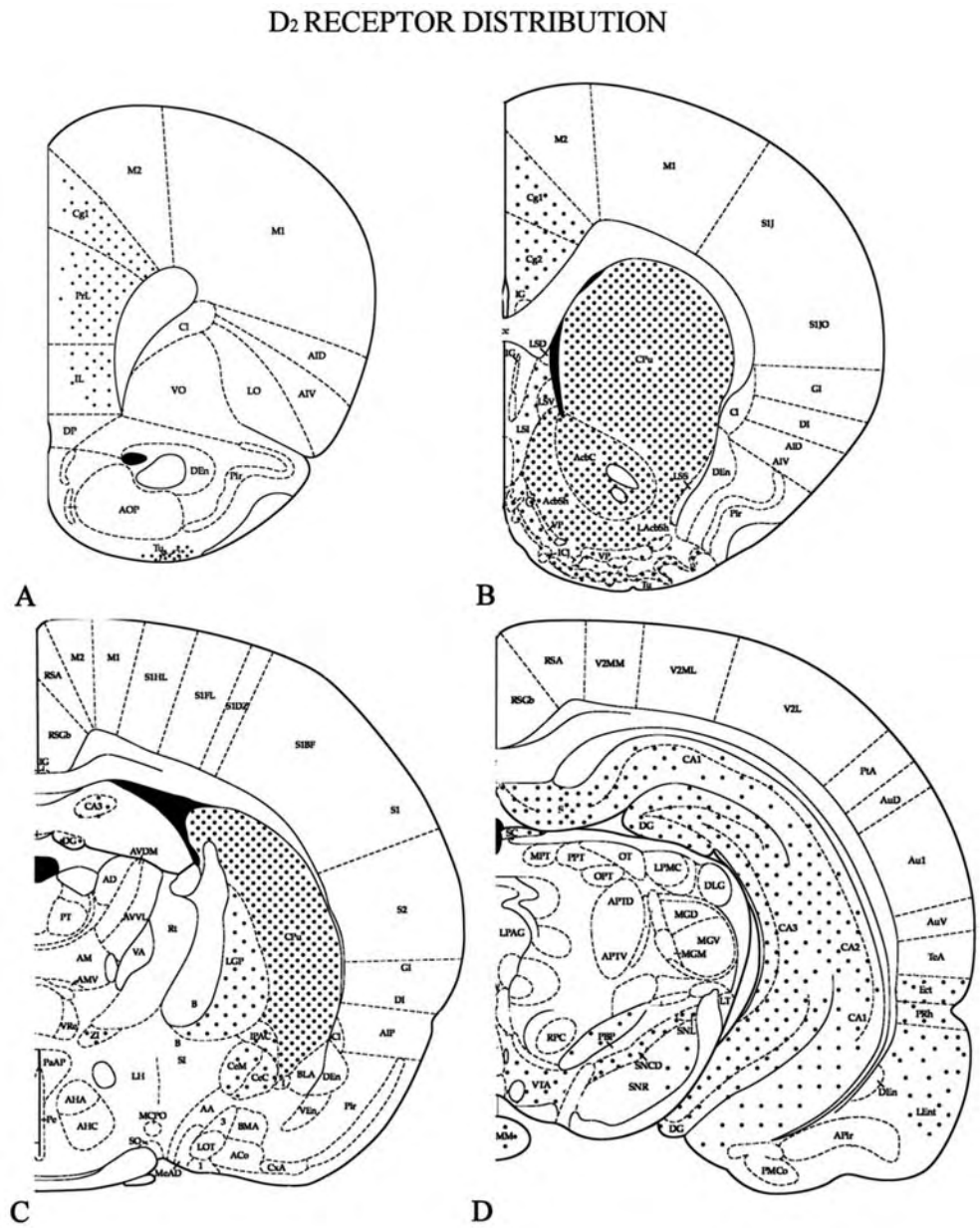

Fig. 25. Distribution of D_2 receptor in representative sections of the rat brain. See the legend to Fig. 22 for further details.

The D_2 receptor distribution parallels, in general, the distribution of the D_2 transcript (Mansour et al., 1990; Meador-Woodruff et al., 1991a; Weiner et al., 1991). Good correspondence between the receptor binding sites and D_2 mRNA was found in the prefrontal and cingulate cortex, NAc, olfactory tubercle, CPu, GP, SNc, VTA and in the

intermediate lobe of the pituitary. Lack of correspondence was instead found in the islands of Calleja, where high receptor level and low mRNA level were detected, and in the hippocampus and zona incerta, in which the mRNA level was higher than that of the receptors (Meador-Woodruff et al., 1989; Mansour et al., 1990; Weiner et al., 1991; Gaspar et al., 1995).

11.2. D_2 RECEPTOR DISTRIBUTION IN THE RAT BASAL GANGLIA

The D_2 receptor distribution in the basal ganglia structures has been extensively studied in relation to the role of these receptors in the control of movements and their involvement in Parkinson's disease, as it has happened for all the other features of the organization and regulation of the dopaminergic innervation of the striatum.

The D_2 receptors are evenly distributed in the CPu with a medial to lateral gradient, so that the lateral portion of the CPu exhibits higher D_2 receptor concentration than the medial portion (Joyce et al., 1985; Fisher et al., 1994).

Immunohistochemical analysis with specific antibodies and in situ hybridization studies showed that D_2 receptor protein and mRNA are located in about 50% of the medium sized neurons and in about 80% of the large cholinergic interneurons (Bouthenet et al., 1987; Le Moine et al., 1990a).

Immunohistochemical studies have shown that in striatal neurons D_2 receptors are concentrated in dendrites and perikarya having the characteristics of either spiny projection neurons or aspiny interneurons (McVitte et al., 1991; Delle Donne et al., 1997). In spiny neurons, D_2 receptors are contained in the spine head within the neuropil more than in the somata (Levey et al., 1993; Fisher et al., 1994; Sesack et al., 1994). Postsynaptic densities are present at asymmetrical striatal synapses in spine head labeled with D_2 receptor antibodies (Levey et al., 1993). D_2 immunoreactivity is also present in preterminal elements as well as in terminal boutons forming symmetrical more than asymmetrical synapses (Levey et al., 1993; Hersch et al., 1995; Wang and Pickel, 2002). Ultrastructural localization of D_2 immunolabeling suggests that the varicose portion of terminals contains little immunoreactivity, whereas preterminal axons contain high D_2 immunolabeling (Sesack et al., 1994; Yung et al., 1995).

D_2 immunolabeling present on DA nerve terminals is consistent with the presence of D_2 autoreceptors. A high proportion of immunoreactivity was also detected on membranes at non-synaptic sites (Yung et al., 1995). D_2 receptors in the SN–CPu system, therefore, are strategically located in order to subserve: (i) autoreceptor functions at the level of dendrites in the midbrain and on the presynaptic axon terminal, (ii) presynaptic functions on nondopaminergic terminals, (iii) postsynaptic actions on CPu spiny dendrites.

An issue of critical importance for the understanding of striatal functions is the localization of D_2 receptors in the striatal medium-sized projection neurons. Several studies using receptor antibodies or in situ hybridization have evidenced a segregation of D_2 receptor on the enkephalin-containing indirect striato-pallidal-nigral pathway and of D_1 receptors on the direct dynorphin/substance P striatonigral pathway (Gerfen et al., 1990; Le Moine et al., 1990b, 1991; Le Moine and Bloch, 1995; Hersch et al., 1995; Yung et al., 1995; Maltais et al., 2000). However, other reports have proposed that all striatal neurons express both the D_1 receptors and the D_2 receptors or that they coexist in variable proportions (Ariano et al., 1992; Surmeier et al., 1992; Aizman et al., 2000) (see also Section 6.1).

In the SN, D_2 receptors are located in the ventral SNr and SNc, both on perikarya and dendrites (Levey et al., 1993; Yung et al., 1995). Lesions of the DA nigrostriatal pathway with 6-hydroxydopamine decreased D_2 receptor binding in the SNc, indicating that D_2 autoreceptors are present in SNc (Morelli et al., 1987, 1988; Filloux et al., 1987a). Moreover, recent studies have shown that the targeted deletion of the D_2 receptor gene in knockout mice leads to total loss of the DA inhibitory effects on both SN DA neuron firing and striatal DA release, indicating that D_2 receptor is the major DA autoreceptor (Tan et al., 2003).

Figure 23 illustrates the features presence of D_2 receptor immunoreactivity in the striatum and in the SN.

11.3. D_2 RECEPTOR DISTRIBUTION IN THE RAT CEREBRAL CORTEX

In the neocortex, a main target of DA neurotransmission is the frontal cortex, where it could mediate effects of DA on psychiatric disorders, as shown by studies on antipsychotic drugs which all bind D_2 receptors.

In the medial prefrontal cortex, D_2 receptor binding localization, evaluated with fluorescently coupled ligands, revealed the presence of D_2 receptor in cell bodies of layers II–VI, with the highest density in the deep layers V and VI. The laminar distribution of receptors is similar to that of mesocortical DA afferents (see Section 8.2), suggesting that D_2 receptors are functionally related to these inputs. D_2 receptors were found to be mainly located in cells with a size range overlapping with both large interneurons and small pyramidal cells, consistent with a localization in neuronal populations different from those expressing D_1 receptors, which were instead mainly found in cells with a size range overlapping with that for nonpyramidal neurons (Vincent et al., 1993).

D_2 mRNA was found to be present in almost all cortical areas, with greater expression in the medial prefrontal, cingulate and insular cortices, and lower in the motor and parietal cortices. D_2 mRNA was restricted to layer V and to corticostriatal and corticocortical neurons (Gaspar et al., 1995). About 50% of D_2 receptor mRNA was found in GABAergic interneurons expressing parvalbumin (Le Moine and Gaspar, 1998).

11.4. D_2 RECEPTOR DISTRIBUTION IN THE RAT LIMBIC SYSTEM

As mentioned in Section 7, the DA mesolimbic system plays a fundamental role in the rewarding properties of either natural or chemical rewards. The role of D_2 and D_1 receptors in the mediation of these behaviors is complex and not yet clarified, since both the receptors are extensively located in these structures.

In the NAc core and in the olfactory tubercle, similarly to the CPu, D_2 receptors are located in GABAergic neurons coexpressing enkephalin, whereas in the NAc shell D_2 receptors are expressed in neurotensin-containing neurons (Le Moine et al., 1990b; Diaz et al., 1994; Le Moine and Bloch, 1995; Delle Donne et al., 1996). In addition, in both the NAc and the olfactory tubercle an overall co-distribution of D_2 receptor and TH immunoreactivity was found, consistent with the presence of D_2 autoreceptors. In the shell portion of the NAc, D_2 immunoreactivity was detected with similar frequency in terminals and dendritic spines (Delle Donne et al., 1997).

In the olfactory bulb, D_2 receptor immunoreactivity was detected in the glomerular and external plexiform layers; the olfactory nerve also exhibited immunopositivity (Levey et al., 1993).

In the hippocampus, D_2 receptor binding is present in the stratum lacunosum molecolare and all layers of subiculum (Mansour et al., 1990; Levey et al., 1993; Yokoyama et al., 1994). In the presubiculum D_2 receptor is expressed in layer II, whereas the parasubiculum does not contain D_2 receptor. Lower levels of D_2 receptor are present in the CA1 field, and in the piriform, entorhinal and perirhinal cortices. In the entorhinal cortex, layers I and III exhibit the highest density of D_2 receptor, whereas in the perirhinal cortex a trilaminar pattern of D_2 receptor is observed, with the highest levels in the external and deep laminae (Goldsmith and Joyce, 1994).

Autoradiographic studies have shown a laminar distribution of D_2 receptors in register with D_1 receptors: layer with high density of one receptor subtype have low density of the other. The D_2 receptor mRNA, on the other hand, was found in the pyramidal cell layer of the Ammon's horn (the CA1, CA2 and CA3 fields) and in the granule cells of the dentate gyrus (Mansour et al., 1990). A mismatch between DA innervation and D_2 receptors was observed in the hippocampus (Mansour et al., 1990; Kohler et al., 1991).

In the amygdaloid complex, D_2 receptors, evaluated by quantitative autoradiography, showed low density in most nuclei, but a relatively high concentration was detected in the central nucleus, with a medial to lateral gradient (Bouthenet et al., 1987; Scibilia et al., 1992).

11.5. D_2 RECEPTOR DISTRIBUTION IN THE HUMAN AND NONHUMAN PRIMATE BRAIN

D_2 receptor has similar regional distribution in the rat, monkey and human brain.

In the human and monkey brain, the highest D_2 receptor binding density is found in the NAc, olfactory tubercle, caudate nucleus and putamen as well as SNc; lower density is present in the GPe, whereas very low density is present in olfactory bulb, GPi, amygdala and cerebellum (Richfield et al., 1987; Camps et al., 1989; Levey et al., 1993).

In the human hippocampus, the highest binding was found in the molecular layer of the dentate gyrus and subiculum, whereas the binding was lower in CA3 and CA1, and no binding was found in the entorhinal cortex; a trilaminar pattern of D_2 receptor was instead observed in the perirhinal cortex (Goldsmith and Joyce, 1994).

In the neocortex, D_2 receptor was detected with the highest density in layer V of frontal, parietal and occipital areas (Lidow et al., 1991).

A similar pattern of expression of D_2 receptor mRNA was found in humans and in the rhesus monkey in the following order of density: caudate and putamen, claustrum, SNc, pyramidal cell layer of hippocampus, cerebral cortex, amygdala, medial and lateral thalamic nuclei and lateral geniculate nucleus (Meador-Woodruff et al., 1991a; Choi et al., 1995; Gurevich et al., 1997).

In the motor cortex in situ hybridization for D_2 mRNA revealed numerous labeled cells throughout layers II–VI, whereas in the prefrontal, temporal, and occipital cortical fields D_2 mRNA was present at modest levels of expression as compared with mRNA of the other DA receptors (Huntley et al., 1992; Meador-Woodruff et al., 1994b, 1996). In the

prefrontal and temporal cortex, D_2 receptor mRNA was found to be expressed in both superficial (II–III) and deep (V–VI) layers, and a similar pattern of distribution was found for D_2 receptor binding in the temporal cortex (Goldsmith and Joyce, 1996; Meador-Woodroof et al., 1996).

In both the nonhuman primates and humans, D_2 mRNA was found to be homogeneously distributed in the striosomal and matrix compartments of the caudate nucleus and putamen, with no medial to lateral gradient. At variance with D_1 receptors, D_2 receptors were also detected in large, putatively cholinergic neurons. In the ventral portion of the striatum, NAc and olfactory tubercle, D_2 receptor mRNA distribution was sparser than in to the dorsal striatum, with islands of tightly packed small cells (Richfield et al., 1987; Huntley et al., 1992; Rappaport et al., 1993; Meador-Woodruff et al., 1996).

The GPi did not exhibit any detectable D_2 receptor binding, whereas in the GPe (equivalent, as mentioned above, to the GP in the rat), the D_2 receptors were present, although at low levels. The SNc was the only portion of the SN containing D_2 receptor (Richfield et al. 1987; Rappaport et al. 1993).

As for the rat, a mismatch between the pattern of distribution of D_2 receptors and dopaminergic innervation was found (Goldsmith and Joyce, 1994).

12. D_3 RECEPTORS

12.1. OVERVIEW OF D_3 RECEPTORS

For more than a decade, the actions of DA were attributed to the D_1 and D_2 receptors, until the presence of a new DA receptor, belonging to the D_2-like family, was demonstrated by Sokoloff et al. (1990). The cloning of this receptor, which was named D_3, indicated a high homology with transmembrane segments I and II of the D_2 receptor, suggesting that the D_3 receptor is encoded by a gene homologous to the D_2 gene.

Similar to the D_2 and D_4 receptors, the D_3 receptor inhibits cAMP accumulation through coupling to G proteins. In addition, the D_3 receptor inhibits Ca^{2+} currents and promotes mitogenesis, probably via tyrosine phosphorylation and activation of mitogen-activated protein kinases (Sokoloff and Schwartz, 2003).

The expression of the D_3 receptor in the brain is, in general, several times lower than that of the D_2 receptor; however, the affinity of DA is higher for the D_3 receptor than for the D_2 receptor.

In contrast to studies on D_1 and D_2 receptors, due to the relative lack of selective D_3 drugs the majority of studies on D_3 receptor distribution have been performed with in situ hybridization rather than with receptor binding autoradiography.

D_3 receptors are characterized by their abundance in limbic areas, whereas the presence of these receptors in motor or other cortical areas is limited (Sokoloff et al., 1990). A report by Levesque et al. (1992), utilizing the D_3 receptor agonist [^{3}H] 7-OH-DPAT, showed that the highest number of D_3 receptor binding sites could be found in the NAc, olfactory tubercle and archicerebellum. This report was confirmed by several studies on D_3 mRNA, which demonstrated that the highest level of receptor transcript was present in the ventral striatal complex (NAc, olfactory tubercle and islands of Calleja), SN and archicerebellum (Bouthenet et al., 1991; Diaz et al., 1995).

Detailed studies on the rat brain reported a wider distribution of the D_3 mRNA. In particular, high density of the D_3 transcript was detected in the medial mammillary

bodies, in other hypothalamic structures, such as the paraventricular hypothalamic nucleus, as well as in the Purkinje cells of lobules 9 and 10 of the cerebellum.

Medium-high density of D_3 mRNA was described in the septal area, in the medial division of the bed nucleus of the stria terminalis, in the nuclei of the horizontal and vertical limbs of the diagonal band, in the nucleus gelatinous and paracentral nucleus of the thalamus, in the medial and ventral lateral geniculate nuclei, and in the lateral portion of the SNc.

Medium-low density of D_3 mRNA was found in the agranular insular, fronto-parietal, temporal and occipital cortices, in the hippocampal formation, amygdaloid complex, in thalamic nuclei (anteroventral, anterodorsal, laterodorsal, ventroposterolateral and centromedial nuclei), in the lateral habenula, CPu, STh, VTA, paraventricular and ventromedial hypothalamic nuclei, and superior colliculus (Bouthenet et al., 1991; Landwehrmeyer et al., 1993b; Diaz et al., 1995, 2000). No receptor signal could be detected in the pituitary gland (Sokoloff et al., 1990).

Except for the *hilar* region of the islands of Calleja, the lateral habenula and the Purkinje cells of cerebellum, D_3 mRNA matched the distribution of the receptor (Diaz et al., 2000). In the archicerebellum, in fact, D_3 mRNA was found to be expressed in the Purkinje cells of lobules 9 and 10, whereas D_3 binding sites were contained in the molecular layer surrounding the Purkinje cell layer of lobule 10 and in the ventral molecular layer of lobule 9 (Levant et al., 1993; Diaz et al., 1995).

Figure 26 depicts the relative density of dopamine D_3 receptor at selected representative levels of the rat brain.

12.2. D_3 RECEPTOR DISTRIBUTION IN THE RAT BASAL GANGLIA

A low density of D_3 receptors was found in the CPu, with the exclusion of the dorsolateral portion where D_3 receptor mRNA was hardly detected (Bouthenet et al., 1991).

In the lateral part of the SNc, VTA and A8 retrorubral field, all the TH-positive cells displayed D_3 receptor immunoreactivity; in contrast, some D_3-positive cells were TH-immunonegative (Diaz et al., 2000). These results support the initial finding that D_3 receptors act as autoreceptors (Sokoloff et al., 1990), as well as the report, based on the study of D_3 receptor mRNA, in which the SNc lateral part was shown to express D_3 receptor transcript (Bouthenet et al., 1991). Electrophysiological studies, however, showed that autoreceptor functions are maintained in D_3 receptor-knock out mice, indicating that the D_2 receptor is the major autoreceptor (Koeltzow et al., 1998).

12.3. D_3 RECEPTOR DISTRIBUTION IN THE RAT LIMBIC SYSTEM

The most relevant contribution of D_3 receptors to the DA functions is related to the actions of these receptors in the limbic system.

In the NAc, D_3 receptor binding and gene transcript are mainly found in medium-sized neurons of the rostral pole and ventromedial shell. In the ventromedial shell, about 60% of D_3 receptor-expressing neurons are neurotensin-positive (Diaz et al., 1995). In the NAc shell and core, D_3 receptor mRNA is coexpressed with either D_1 receptor or D_2 receptor mRNA in a subpopulation of substance P-containing and enkephalin-containing neurons, respectively. A significant part of NAc neurons, however, express either D_1 or D_2 receptors without coexpression with D_3 receptors (Le Moine et al., 1996). In the NAc shell, selective polyclonal antibody towards D_3 receptor revealed a punctate distribution at the

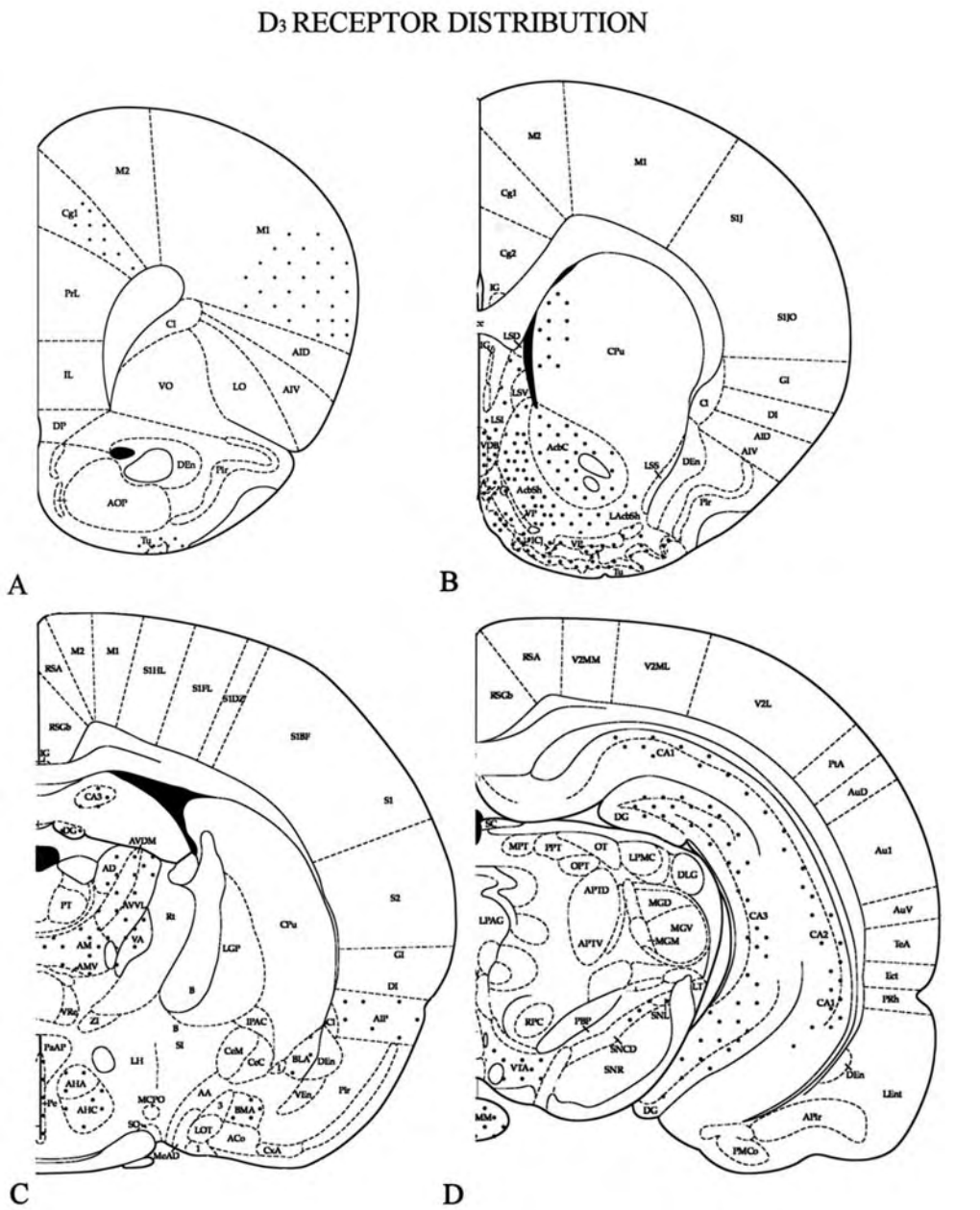

Fig. 26. Distribution of D_3 receptor in representative sections of the rat brain. See the legend to Fig. 22 for further details.

plasma cell membrane and within the cytoplasm but not in the cell nucleus. D_3 immunoreactivity did not overlap with synaptophysin immunoreactivity, indicating that D_3 receptors are not present in the vicinity of synaptic boutons (Diaz et al., 2000). Therefore, similar to D_1 and D_2, D_3 receptors are also mainly extrasynaptic, a classical

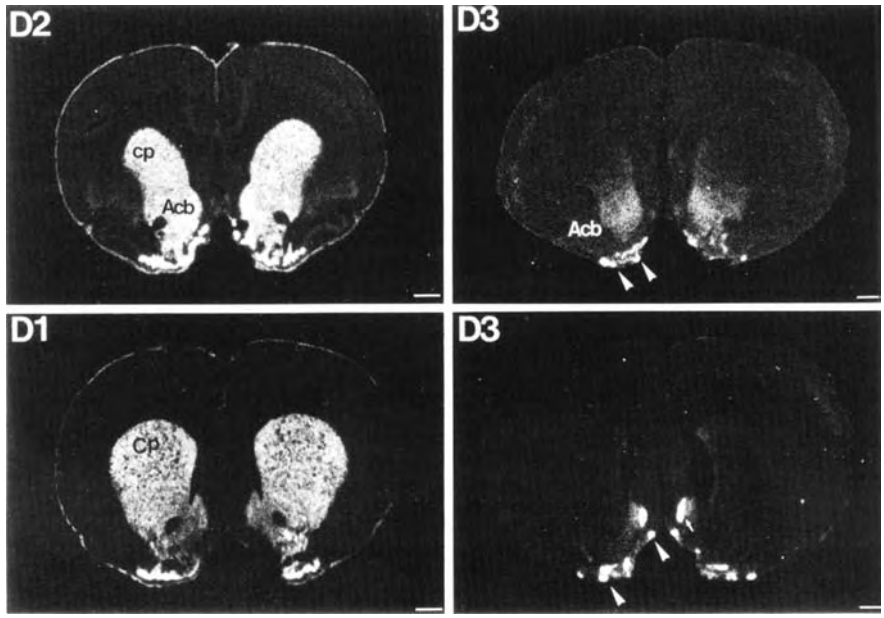

Fig. 27. Comparison of the general topography of the expression of D_1, D_2 and D_3 receptor genes in the dorsal and ventral striatum, as shown by in situ hybridization. Note the overall similar distribution of the D_1 and D_2 mRNAs in the caudate-putamen (cp) and nucleus accumbens (Acb); in contrast, note the restricted distribution of D_3 mRNA in the nucleus accumbens, and the major (arrow) and minor (arrowheads) islands of Calleja. Scale bar. 1 mm. Reproduced with permission from LeMoine and Bloch (1996).

situation observed for neuromodulators which act at some distance from their site of release.

In the islands of Calleja, D_3 binding sites and mRNA are located on the entire population of granule cells, which establish sparse contacts with dopaminergic axons (Diaz et al., 1995). An extensive coexpression of D_1 and D_3 mRNAs was found in the granule cells of the island of Calleja major, which express substance P mRNA (Ridray et al., 1998).

Several areas express both the D_2 and D_3 receptors and the mRNAs; however, overlap of the two receptors is rarely found (Bouthenet et al., 1991). A comparative study of D_3 and D_2 mRNAs showed that in the islands of Calleja, where the D_3 message is at the highest level, no D_2 receptor message is detectable (Bouthenet et al., 1991; Landwehrmeyer et al., 1993b) (Fig. 27). In the bed nucleus of the stria terminalis, the cells of the medial division express selectively D_3 receptors, whereas in the lateral and ventral divisions only D_2 receptors are expressed (Bouthenet et al., 1991).

The D_3 receptor was also found in the hippocampal formation (granule cell layer of the dentate gyrus) and in the amygdaloid complex (anterior, basomedial and medial nuclei). In the posterior hypothalamus, D_3 receptor was found to be expressed in the medial mammillary nucleus (Bouthenet et al., 1991).

12.4. D_3 RECEPTOR LOCALIZATION IN THE HUMAN AND NONHUMAN PRIMATE BRAIN

The overall distribution of D_3 receptor in the human and rat brain appears similar in terms of localization and abundancy (Hall et al., 1996). In both the species the D_3 message was

found to be highest in limbic areas (islands of Calleja and ventral striatum/NAc), and medium-high in some thalamic nuclei (such as the anteromedial and mediodorsal nuclei) and in the dentate gyrus of the hippocampus (Suzuki et al., 1998; Gurevich and Joyce, 1999). Differences are, however, reported in the neocortex: in the rat, D_3 mRNA was detected in all cortical layers, whereas in the human brain a laminar prevalence was found. Thus, D_3 transcript was found in the prefrontal and temporal cortex; but the highest level was detected in cingulate cortex and striate region of occipital cortex in both superficial layers and deep layers IV and VI (Meador-Woodruff et al., 1996; Suzuki et al., 1998). In most neocortical areas the highest D_3 mRNA level was found in the superficial and intermediate layers II and IV (Suzuki et al., 1998).

Marked differences between the rat and human brain are represented by the almost total absence of D_3 receptors in the human VTA and the relatively high number of D_3 receptors and mRNA in the human dorsal striatum (Landwehrmayer et al., 1993a; Meador-Woodruff et al., 1994a; Gurevïch and Joyce, 1999), a finding confirmed in nonhuman primates (Hurley et al., 1996).

Receptor binding studies in the human brain with D_2 and D_3 receptor ligands, showing the relative ratio of D_3 and D_2 receptors, reported a prevalence of D_3-like receptor binding in the NAc and ventral putamen with a rostrocaudal gradient (Murray et al., 1994; Suzuki et al., 1998; Gurevich and Joyce, 1999). In these areas, D_3-like binding was found to be intense in AchE-poor striosomes, whereas D_2-like binding was highest in the matrix compartment (Murray et al., 1994). The proportion of D_3 versus D_2 receptors was highest also in the septum, islands of Calleja, nucleus basalis, GPi, ventral pallidum and central amygdala (Murray et al., 1994).

Other differences between the human and the rat brain consist in the high degree of coexpression of D_2 and D_3 mRNAs in the same neuronal population, suggesting a functional convergence of the two receptors in many regions of the human brain (Gurevich and Joyce, 1999). Moreover, it appears that the rat and human nigrostriatal (SNc) and mesocorticolimbic (VTA) DA systems may be differently autoregulated, since in humans only the projections arising from the SNc, but not those from the VTA, express D_2 and D_3 autoreceptors (Meador-Woodruff et al., 1994a).

The abundance of the D_3 receptor levels in limbic-related regions supports the view that D_3 receptors may be involved in the modulation of emotional and cognitive processes. However, it is also evident that the D_3 system extends beyond limbic and limbic-related structures, contributing to the complex of behaviors which characterize both emotional and cognitive processes (Sokoloff and Schwartz, 2003).

13. D_4 RECEPTORS

13.1. OVERVIEW OF D_4 RECEPTORS

The D_4 receptor, cloned by Van Tol et al. in 1991, exhibits a homology of 41% and 39% with the D_2 and D_3 receptors, respectively. The principal transduction mechanism of the D_4 receptor is the inhibition of adenylyl cyclase; the D_4 receptor, however, also stimulates Na^+/H^+ exchange and potentiates stimulated arachidonic acid release (Chio et al., 1994; McHale et al., 1994).

Little is known about the physiological role of the D_4 receptor, except for some reports on D_4 receptor role in behavioral sensitization (Feldpausch et al., 1998) and on reversal of

prepulse inhibition, a method to assess antipsychotic activity of drugs (Mansbach et al., 1998). Moreover, a role of the D_4 receptor in schizophrenia has been suggested by studies on postmortem tissue samples from schizophrenic patients, and on the basis of the efficacy of clozapine, an atypical antipsychotic drug, which has high affinity for D_4 receptors (Seeman et al., 1993).

Similarly to the D_3 and $D_{1B/5}$ receptors, the localization of the D_4 receptor in the central nervous system was mostly evaluated through the localization of its mRNA and through studies using specific antibodies. The level of D_4 receptor expression in the CNS was found, in general, to be lower than that of the D_2 receptor. D_4 receptor expression was found to be higher in limbic and cortical areas than in motor areas, suggesting a preferential involvement of these receptors in affective behavior and cognition (Civelli, 2003).

High D_4 receptor concentration has been described in the rat frontal and parietal cortex; on the other hand, medium-high concentration of D_4 receptor has been reported in the NAc, CPu, SNc, as well as in the hippocampus, entorhinal cortex, and medium-low concentration in the olfactory tubercle, GP, thalamus, supraoptic nucleus of the hypothalamus, cerebellum, and pituitary gland (Ariano et al., 1997b; Defagot et al., 1997; Mauger et al., 1998).

In the rodent brain, D_4 mRNA has a relatively restricted pattern of expression, being localized in the frontal cortex, in the olfactory bulb, hypothalamus and thalamus; in the retina, D_4 mRNA is expressed in the photoreceptor cell layer and in the inner nuclear and ganglion cell layers (Cohen et al., 1992; O'Malley et al., 1992).

Figure 28 depicts the relative density of the D_4 receptor in selected representative levels of the rat brain.

13.2. D_4 RECEPTOR DISTRIBUTION IN THE BASAL GANGLIA

The D_4 receptor has a scattered distribution in mouse CPu (Mauger et al., 1998) and appears to be more abundant in the striatal patches than in the matrix of the rodent brain (Rivera et al., 2002b). The D_4 receptor was found in both neuropil and cell bodies, and was mainly localized in the dendritic shaft and spines (Rivera et al., 2002b). The D_4 receptor was also found in neurons of the rodent GP, where it reduces GABAergic currents (Mauger et al., 1998; Shin et al., 2003).

13.3. D_4 RECEPTOR DISTRIBUTION IN THE CEREBRAL CORTEX

The D_4 receptor has been described in layers II–VI of both the frontal and the piriform cortex of the mouse brain, with the highest concentration in layer II. D_4 receptors are distributed in somata and proximal processes of pyramidal neurons. In cortical regions, the levels of D_4 receptors were found to be higher than those of D_2 and D_3 receptors (Ariano et al., 1997; Mauger et al., 1998).

13.4. D_4 RECEPTOR DISTRIBUTION IN THE LIMBIC SYSTEM

In the rat limbic system, the highest D_4 receptor concentration of was found in the NAc and in the hippocampal formation (the fields CA1, CA2, CA3, and dentate gyrus), whereas the olfactory tubercle and the entorhinal cortex showed a lower concentration (Defagot et al., 1997).

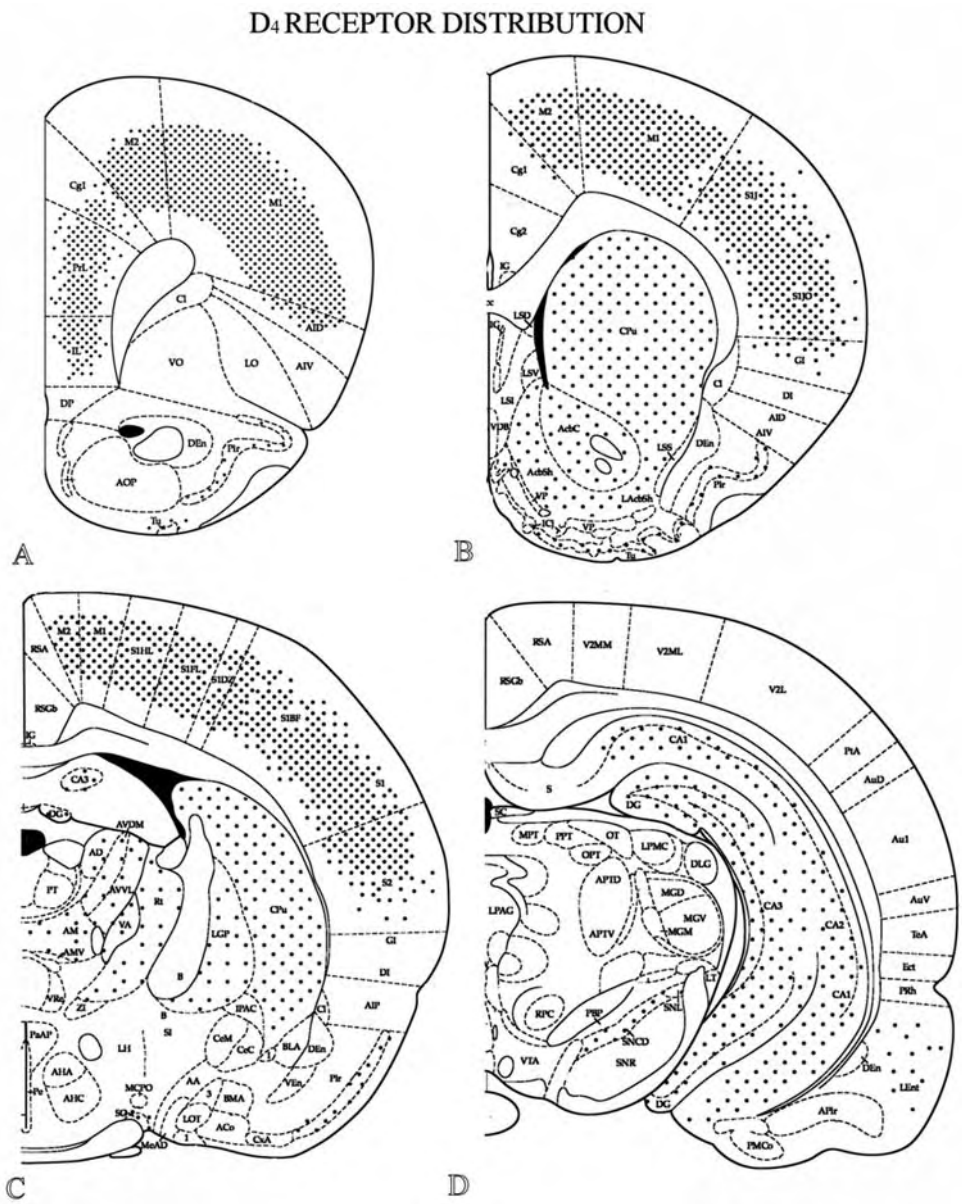

Fig. 28. Distribution of D_4 receptor in representative sections of the rat brain. See the legend to Fig. 22 for further details.

Studies at the ultrastructural level in the rat NAc shell showed that 65% of D_4 receptors were contained in axons and axon terminals, in plasma and vesicular membranes, while only 22% were located in dendrites and dendritic spines which received input from TH-positive terminals (Svingos et al., 2000). The labeled terminals formed occasionally asymmetrical synapses, and only about 17% of them exhibited TH immunoreactivity. In the NAc, therefore, the D_4 receptor appears to be involved in presynaptic rather than postsynaptic functions (Svingos et al., 2000).

13.5. D_4 RECEPTOR LOCALIZATION IN THE HUMAN AND NONHUMAN PRIMATE BRAIN

In the primate brain, a high concentration of the D_4 receptor was detected in the cerebral cortex, hippocampus, thalamic reticular nucleus, GP and SNr (Mrzljak et al., 1996). Similar to rodents, in the primate striatum the D_4 receptor appears to be more abundant in striosomes than matrix (Rivera et al., 2002b).

D_4 mRNA was found at the highest level in the human prefrontal cortex, and the trasncript was equally high in the human retina; high D_4 mRNA was found in the human amygdala, dentate gyrus and hippocampal CA2 field, entorhinal cortex, medial temporal lobe, hypothalamus, thalamus, cerebellum, and pituitary gland, whereas no D4 mRNA was detected in the VTA and SN (Meador-Woodruff et al., 1994a; Matsumoto et al., 1995).

14. $D_{1B/5}$ RECEPTORS

14.1. OVERVIEW OF $D_{1B/5}$ RECEPTORS

The postulated presence of multiple D_1 receptors was ascertained by Tiberi et al. (1991) and Sunahara et al. (1991), who showed, in the rat brain, the presence of a DA receptor structurally and functionally similar to the D_1 receptor. This was then termed D_{1B} receptor in rodents and D_5 receptor in primates.

The localization of D_{1B} and D_5 receptors by receptor binding autoradiography has been hampered by the lack of high affinity $D_{1B/5}$ specific ligands. Therefore, in situ hybridization studies of the receptor mRNA and immunohistochemical studies with specific anti-$D_{1B/5}$ antibodies have been used to map the distribution of $D_{1B/5}$ receptors in the rat, monkey and human brain (Niznik et al., 2003).

As compared to the very high concentration of the D_1 receptor, D_{1B} mRNA and D_{1B} receptor in the rat were found in a lower concentration in the frontal cortex, NAc, olfactory tubercle, and striatum. In contrast, higher levels than D_1 were found in distinct layers of the hippocampus, as well as in the mammillary nuclei and anterior pretectal nuclei (Tiberi et al., 1991; Meador-Woodruff et al., 1992; Ariano et al., 1997b). The parafascicular nucleus of the thalamus was found to be particularly rich in D_{1B} receptors.

Similarly, D_5 mRNA exhibited a lower concentration than D_1 mRNA in the primate striatum. High levels of D_5 mRNA were found in the frontal cortex, in the SN, thalamus and dentate gyrus of the hippocampal formation (Beischlag et al., 1995; Choi et al., 1995). Although the D_1 receptor is in general more abundant than D_5 receptor, in cholinergic neurons of the basal forebrain, D_5 receptors were found to be present at a higher concentration than D_1 receptors (Beischlag et al., 1995; Bergson et al., 1995b).

As in the earlier distributional maps, Fig. 29 depicts relative density of dopamine D_{1B} receptor in selected representative levels of the rat brain.

14.2. $D_{1B/5}$ RECEPTOR DISTRIBUTION IN THE BASAL GANGLIA

In the primate striatum, D_5 mRNA does not exhibit the striosomal compartmentalization found for D_1 mRNA, and the levels are roughly equal in the ventral and dorsal striatum (Rappaport et al., 1993). Moreover, it was found that, similar to D_1 receptors but to a

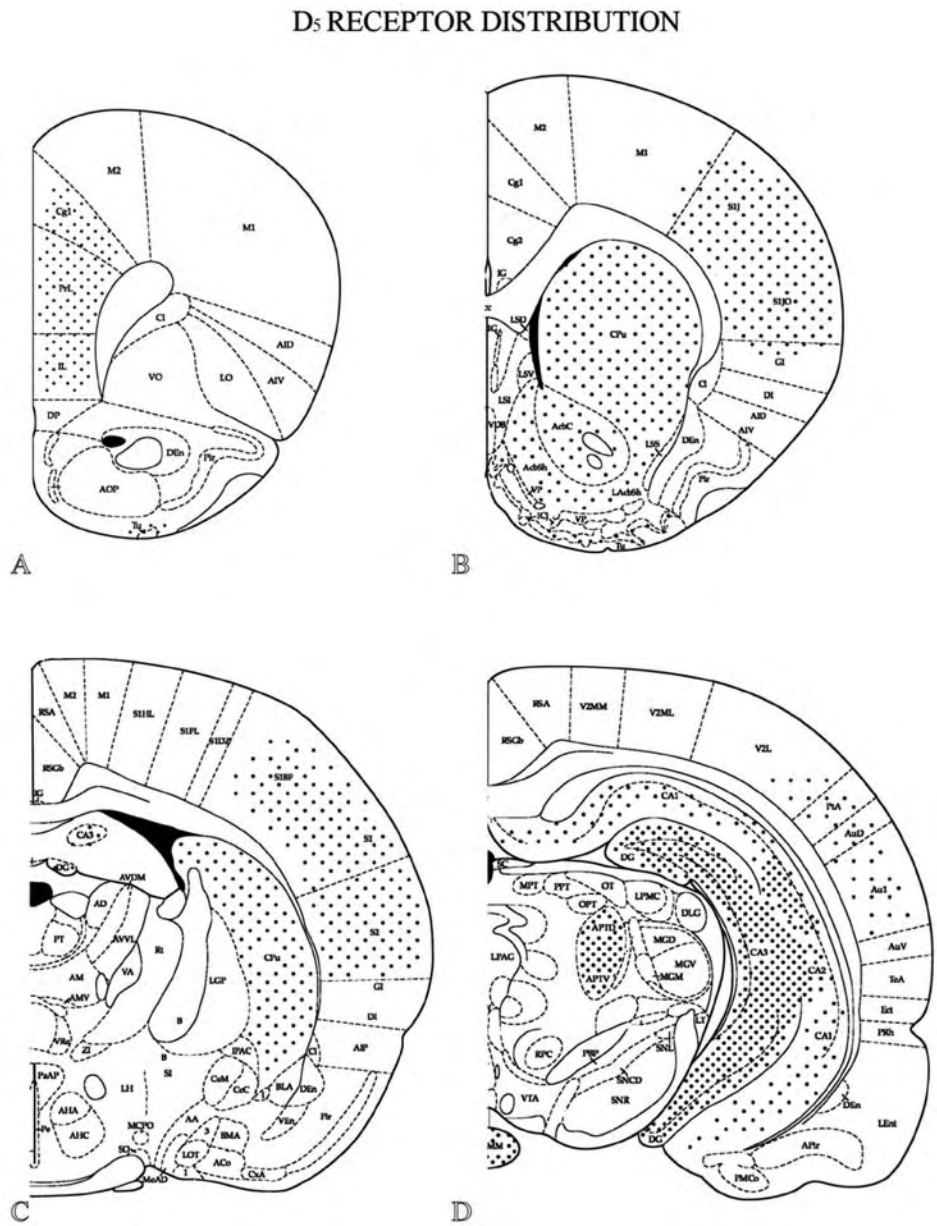

Fig. 29. Distribution of $D_{5/1B}$ receptors in representative sections of the rat brain. Although, as specified in the text, the receptor nomenclature is D_{1B} in rodents and D_5 in primates, the map has been labeled as D_5 to avoid any confusion with Fig. 22. See the legend to Fig. 22 for further details.

lesser extent, D_5 receptors are contained in striatal medium-sized spiny neurons, whereas, differently from D_1 receptors, D_5 receptors are present in large aspiny neurons (Bergson et al., 1995a). Ultrastructural studies have demonstrated that the D_5 receptor is predominantly present in the dendritic shaft of the projection neurons (Bergson et al., 1995b).

In rat CPu, the D_{1B} receptor, although at low concentration, is present in projection neurons of both the direct and indirect pathways (Rivera et al., 2002a).

In the primate SNr, the D_5 receptor is present in a few scattered cell bodies but is undetectable in the neuropil (Bergson et al., 1995b).

The different cellular and subcellular localization of D_5 receptors and D_1 receptors suggests that the two receptors are associated with different circuits and may play distinct roles in synaptic transmission.

14.3. $D_{1B/5}$ RECEPTOR DISTRIBUTION IN THE NEOCORTEX

Similar to the D_5 receptor, the D_{1B} receptor is expressed in the neocortex, with highest expression in frontal, parietal and temporal areas of the rat cortex (Meador-Woodruff et al., 1994b; Niznik et al., 2003). Moreover, similarly to D_1 mRNA, D_5 mRNA is most abundant in discrete cortical layers (II, IV, VI) (Beischlag et al., 1995). D_1 and D_5 mRNA are frequently coexpressed in pyramidal neurons, with predominant localization in the dendritic shaft of these cells (Bergson et al., 1995b).

14.4. $D_{1B/5}$ RECEPTOR DISTRIBUTION IN THE LIMBIC SYSTEM

In the primate hippocampus the D_5 receptor was found to be frequently coexpressed with the D_1 receptor in pyramidal neurons. However, while the D_1 receptor is prominent in dendrites and dendritic spines, the D_5 receptor is mostly localized in the dendritic shaft (Bergson et al., 1995b). In the human brain, D_5 mRNA is present in the hippocampus and subicular complex.

15. CONCLUDING REMARKS

This chapter demonstrates that the neural networks in which midbrain dopaminergic neurons are inserted and in which DA exerts its action are among those which have raised the greatest interest since the birth of neuroscience. The acceleration of knowledge in the past decades indicates that a lot is still to come to put in place the pieces of the big basal ganglia puzzle, and in particular the pieces of DA regulation in this puzzle. The wealth of data accumulated until now on the DA action on closed and open loops in the basal ganglia, modules of information processing, basal ganglia action-gating circuits, state-setting modulatory circuits provides undoubtedly an example of an exciting scientific endeavor. Acting at the interface between the limbic and motor systems, dopaminergic circuits are involved in an integrative role in the manifestation of motor behavior and its motivational aspects, in a variety of cognitive functions including the generation of context-dependent behaviors, in reward mechanisms subserving biological key functions for survival, as well as addictive phenomena.

Dopaminergic neurons and DA receptors are affected in major neurodegenerative disorders, whose etiopathogenesis is still unknown. The degeneration of DA-containing midbrain neurons is a hallmark of Parkinson's disease, the second most common human neurodegenerative diseases after Alzheimer's dementia, and the most common neurodegenerative movement disorder. Approximately 1% of the population older than 65 years suffers from Parkinson's disease, whose incidence increases markedly with age and therefore in the aging population of developed countries, and 95% of the cases of

Parkinson's disease are sporadic (Bossy-Wetzel et al., 2004; Vila and Przedborski et al., 2004). The dopaminergic hypothesis of schizophrenia, based largely on the efficacy of neuroleptic drugs, is still exerting a high impact on the studies on this debilitating, chronic mental disorder which affects about 1% of people (Freedman, 2003; Carlsson et al., 2004; Siever and Davis, 2004). Drug abuse and dependence, in which dopaminergic brain systems and DA regulation exert a key role, are the major plagues of modern societies.

The etiopathogenesis and therapy of these and other major neurological and psychiatric disorders in which DA is involved represent a great challenge for basic and clinical neuroscience in the new century. Future knowledge on the central dopaminergic systems has great chances to lead to progresses in this field, as it did in the past and is doing at present.

16. ABBREVIATIONS

AChE	acetylcholinesterase
BNST	bed nucleus of the stria terminalis
CCK	cholecystokinin
CPu	caudoputamen
Cx	connexin
DA	dopamine
EP	entopeduncular nucleus
GABA	γ-amino-butyric acid
GPe	globus pallidus, external segment
GPi	globus pallidus, internal segment
HRP	horseradish peroxidase
MD	mediodorsal nucleus of the thalamus
MHC	major histocompatibility complex antigens
MPTP	1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine
NAc	nucleus accumbens
NO	nitric oxide
NOS	nitric oxide synthase
REM	rapid eye movement (sleep)
RRA	retrorubral area
RT-PCR	reverse transcriptase-polymerase chain reaction
SDF-1	stromal cell-derived factor 1 (also designated as CXCL12)
SN	substantia nigra
SNc	substantia nigra pars compacta
SNl	substantia nigra pars lateralis
SNr	substantia nigra pars reticulata
STh	subthalamic nucleus
TH	tyrosine hydroxylase
VTA	ventral tegmental area

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18. REFERENCES

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CHAPTER II

Signal transduction of dopamine receptors

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1. INTRODUCTION

More than 40 years after having been identified as a neurotransmitter, dopamine still remains widely studied because it regulates neural mechanisms having medical implications of utmost importance. Historically, the major discovery that gave rise to an intense activity in this research area was the discovery that dopamine neurons degenerated in the substantia nigra of patients suffering from Parkinson's disease. Rapidly, this degeneration was shown to account for the symptoms of the disease and L-DOPA, the precursor of dopamine able to cross the blood-brain barrier, was recognized as the most efficient drug for treating patients. In Huntington's disease, dopaminoceptive neurons degenerate in the striatum and the drugs blocking dopamine receptors have some beneficial effects at the beginning of the illness. Dopamine neurotransmission is also of major interest in psychiatric disorders. Virtually all the antipsychotic drugs are antagonists at dopamine receptors supporting the hypothesis that alterations in dopamine transmission contribute to the etiology of schizophrenia, or at least to the emergence of its symptoms. Conversely, attention deficit hyperactivity disorder can be treated by drugs stimulating dopamine transmission in the brain. Finally, the common effect of most illicit drugs of abuse, including psychostimulants, opiates or cannabinoids, as well as licit drugs, such as nicotine or alcohol is to stimulate the dopamine neurotransmission especially at the level of the nucleus accumbens.

The largest population of dopamine cell bodies in the CNS is located in the ventral mesencephalon in the pars compacta of the substantia nigra and in the ventral tegmental area. Despite their restricted number, only 450,000 in human (German et al., 1983), their axons are exceptionally branched and their projection areas relatively wide in the anterior parts of the brain, a single dopamine neuron contacting about 300–400 target neurons (Schultz, 1998). Some structures innervated by dopamine neurons are classically associated with the extrapyramidal motor system, including the caudate nucleus-putamen which receives an abundant dopamine innervation originating from cell bodies located in the substantia nigra. The other structures which are essentially innervated by neurons issued from the ventral tegmental area are associated with limbic areas, including the nucleus accumbens and the amygdala, as well as several cortical areas linked to the limbic system such as the prefrontal cortex.

The studies on the role of dopamine transmission in the brain led researchers to distinguish two types of functions (Schultz, 1998). First, dopamine neurons facilitate

globally the functions of the brain areas that they innervate (Le Moal and Simon, 1991). Therefore dopamine neurons from the substantia nigra that project towards a structure dedicated to motor functions like the striatum, facilitate some forms of motor behaviors. Such a role accounts for the efficiency of L-DOPA treatment in Parkinsonian patients. The other important function of dopamine neurons appeared clearly when the electrophysiological activity of dopamine neurons was recorded in behaving monkeys. These studies have shown that dopamine neurons are phasically activated when an unexpected reward is offered to the animals, and are on the contrary inhibited when an expected reward is omitted. Dopamine neurons appear to play a key role in reward-directed learning by signaling errors of reward prediction (Waelti et al., 2001). Dopamine neurotransmission would facilitate this type of learning by promoting synaptic plasticity in the neuronal network innervated by dopamine neurons. There is some evidence of this facilitating influence at a cellular level, in experiments of long-term potentiation (LTP) or long-term depression (LTD) on the synapses between cortical afferent and striatal neurons (Berke and Hyman, 2000; Centonze et al., 2001; Hyman and Malenka, 2001; Reynolds and Wickens, 2002).

The functions of dopamine neurons are characterized at physiological or behavioral levels, but little is known about how these functions emerge from the molecular and cellular actions of dopamine. All the cloned receptors for dopamine belong to the family of G protein-coupled receptors. Consequently, the stimulation of these receptors by dopamine does not alter directly the membrane potential of neurons, but activates cascades of intracellular reactions, through specific GTP-binding proteins (G protein) and possibly other proteins, leading to the regulation of a wide array of proteins. Thus, the consequences in the target cells could, in principle, not only vary depending on the receptor type, but also on the components of signal transduction pathways present in the cell. The aim of this chapter is to review the intracellular events generated by the different types of dopamine receptors in dopamine-innervated cells and a special emphasis will be given to the identification of the G proteins responsible for induction of dopamine signaling and to the proteins whose activity is modulated by this signaling.

2. HISTORICAL ELEMENTS

The first intracellular effect mediated by dopamine receptors that has been reported was the stimulation of the production of cyclic AMP (cAMP) in target cells. This effect was originally described in the superior cervical ganglia and the cow retina (Kebabian and Greengard, 1971; Brown and Makman, 1972) and soon afterward, in the CNS, in rat striatum (Kebabian et al., 1972). Antipsychotic drugs were found to block this response (Clement-Cormier et al., 1974; Miller et al., 1974) and this was the first direct evidence supporting the hypothesis proposed by A. Carlsson in the 1960s that the therapeutic actions of neuroleptics result from their ability to block dopamine receptors.

It was later found that some effects of dopamine did not involve stimulation of adenylyl cyclase. Particularly, in the pituitary gland, dopamine was found to inhibit prolactin release without stimulating adenylyl cyclase activity and even by inhibiting it (Spano et al., 1978; De Camilli et al., 1979). Moreover, the antipsychotic drug sulpiride blocked the dopamine-induced release of prolactin in the pituitary gland but was unable to antagonize the dopamine response on adenylyl cyclase activity in the striatum (Trabucchi et al., 1975). These observations led to the hypothesis that the dopamine receptors exist as two

distinct populations, one called D1 able to stimulate adenylyl cyclase and the other called D2 negatively coupled to adenylyl cyclase activity (for a review, see Kebabian and Calne, 1979).

In the following years, research on dopamine receptors confirmed the concept of the D1/D2 classification, and the introduction of gene cloning procedures in the late 1980s has not invalidated this distinction (see Sibley and Monsma, 1992 for a review). Five different dopamine receptors have been cloned in mammals, all these receptors belonging to the superfamily of G protein-coupled receptors with seven transmembrane domains. The detailed comparison of their amino acid sequence, pharmacological profile and biochemical properties has shown that all the dopamine receptor subtypes can be grouped into two categories displaying many similarities with the two initially defined subclasses. Particularly, the distinction based on the signaling events that they initiate in target cells, remains pertinent. The D1 and D5 receptor subtypes which are both positively coupled to the adenylyl cyclase, are now classified as D1-like receptors whereas the D2, D3 and D4 receptor subtypes which are able to inhibit cAMP production, are classified as D2-like receptors (see Missale et al., 1998 for a review).

3. SIGNAL TRANSDUCTION OF D1-TYPE RECEPTORS

When transfected in heterologous cell systems, both the D1 and D5 receptors are able to stimulate the production of cAMP (Dearry et al., 1990; Tiberi et al., 1991). Although other signaling pathways have been described, the cAMP pathway stimulated by D1/D5 receptors remains the most extensively studied and, it has been described in a very interesting manner, in detail, at the level of the dorsal and ventral striatum, the main projection areas for mesencephalic dopamine neurons.

3.1. D1-TYPE RECEPTOR STIMULATION OF cAMP PATHWAYS

3.1.1. Coupling by G proteins

The first demonstration of the implication of a G protein in the signal transduction of D1 receptor appeared when the dopamine responses on cAMP formation, which are lost in purified striatal membranes cleared of cytoplasmic fraction, were recovered following addition of exogenous GTP (Clement-Cormier et al., 1975). Several years later the binding of agonists with D1 receptors was found to be modulated by GTP (Schulz et al., 1985). When the D1 and D5 receptors have been cloned, they were found to belong to the family of receptors coupled to a G protein (Dearry et al., 1990; Grandy et al., 1990; Monsma et al., 1990; Zhou et al., 1990; Tiberi et al., 1991). The G proteins are heterotrimeric proteins associating α, β and γ subunits and those able to stimulate adenylyl cyclase are characterized by the presence of homologous α subunits encoded by either of the two genes, $G\alpha_s$ and $G\alpha_{olf}$. Because $G\alpha_s$ is widely expressed in many cell types including neurons, $G\alpha_s$ was considered during several years to be responsible for the coupling of D1 receptor to adenylyl cyclase in the striatum. In situ hybridization revealed however that the striatum in which the D1 receptors are the densest in the brain, expressed little $G\alpha_s$ but abundant $G\alpha_{olf}$ (Largent et al., 1988; Drinnan et al., 1991). $G\alpha_{olf}$, originally identified in the olfactory epithelium, shares 80% identity in amino acid sequence with $G\alpha_s$ (Jones and Reed, 1989). $G\alpha_{olf}$ was clearly demonstrated to be responsible for coupling D1 receptors to

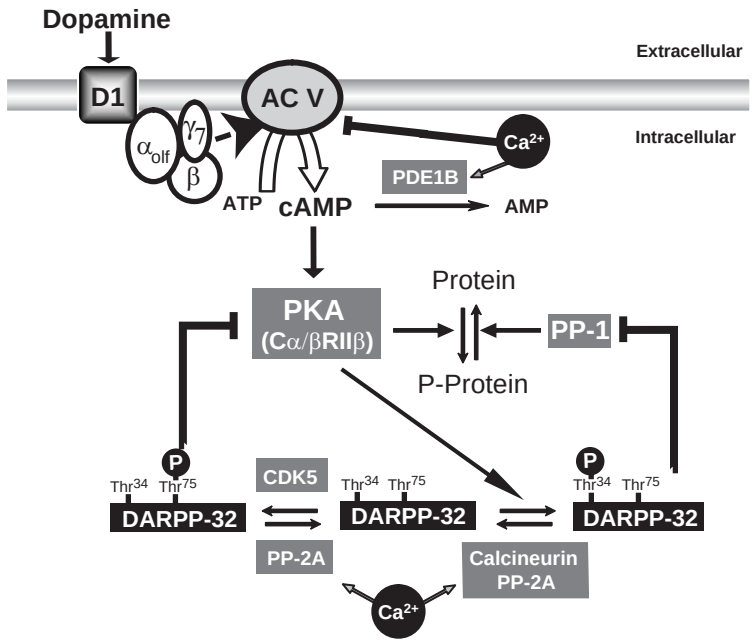

Fig. 1. cAMP pathway in the striatal neurons expressing D1 dopamine receptor. In striatal neurons, the cAMP pathway stimulated by the activation of D1 receptor is highly differentiated, involving specific isoforms of signaling proteins: $G\alpha_{olf}$ and $G\gamma_7$ subunits of G protein, type V adenylyl cyclase (ACV), RIIβ regulatory subunit of PKA, PDEB1 isoform of phosphodiesterase and DARPP-32. DARRP-32 is an important switch in the cascade of protein phosphorylation triggered by activation of D1 receptor. In basal condition, DARPP-32 is phosphorylated on Thr75 by CDK5 and inhibits PKA. Following D1 receptor activation, DARPP-32 is phosphorylated on Thr-34 via PKA activation, becomes a potent inhibitor of PP-1 and consequently stabilizes the phosphorylation state of numerous proteins. Intracellular Ca^{2+} exerts globally an inhibitory influence on the D1 receptor signaling by inhibiting ACV and by activating PDE1B and calcineurin. Ca^{2+} may also activate PP2A by an unknown mechanism (see text for references).

adenylyl cyclase in striatum since the stimulation of cAMP production by D1 receptor activation is virtually abolished in the striatum of $G\alpha_{olf}$ knockout mice (Corvol et al., 2001). The medium-sized spiny neurons, which contain high levels of D1 receptors, express $G\alpha_{olf}$ and very little, if any, $G\alpha_s$ (Herve et al., 1993, 2001). A population of the medium-sized spiny neurons contains few or no D1 receptors, but expresses high levels of $G\alpha_{olf}$. In these neurons, the role of $G\alpha_{olf}$ is to couple A2a receptor to adenylyl cyclase (Kull et al., 2000; Corvol et al., 2001). In many regions outside of the basal ganglia, including the cerebral cortex, $G\alpha_{olf}$ is not expressed and $G\alpha_s$ may couple D1 receptors to adenylyl cyclase, as suggested by the normal D1 responses observed in the cerebral cortex of the $G\alpha_{olf}$ knockout mice (Corvol et al., 2001).

Homozygous $G\alpha_{olf}$ knockout mice displayed several behavioral alterations similar to those observed in D1 receptor knockout mice, including a strong increase in spontaneous locomotor activity, a blockade of cocaine-induced locomotor activity and an impaired c-fos induction by cocaine (Belluscio et al., 1998; Zhuang et al., 2000). However, because $G\alpha_{olf}$ transduces the odorant signals in primary olfactory neurons (Jones and Reed, 1989), the loss of $G\alpha_{olf}$ in these mice gives rise to very severe olfactory deficits, and many mutant mice die in the days following birth because of feeding deficiency (Belluscio et al., 1998). This severe phenotype, which may result from both olfactory impairment and alterations

in dopamine signaling, renders difficult the analysis of effects, related to dopamine neurotransmission alterations.

Heterozygous $G\alpha_{olf}$ +/− mice provide an interesting model for studying the role of $G\alpha_{olf}$ in dopamine actions since the amounts of $G\alpha_{olf}$ in striatal areas appear to be a limiting parameter for the D1 effects on cAMP production (Corvol et al., 2001). In $G\alpha_{olf}$ +/− mice, the levels of $G\alpha_{olf}$ are about half of those in wild type, there are no compensatory changes in $G\alpha_s$, and the activation of adenylyl cyclase by dopamine is significantly lower (Corvol et al., 2001). This is surprising since D1 receptors are far less abundant than $G\alpha_{olf}$ in the striatum (1.3 and 17 pmol/mg protein, respectively). This contrast suggests that a single dopamine-stimulated D1 receptor activates numerous $G\alpha_{olf}$ proteins and that despite its high levels in the striatum $G\alpha_{olf}$ can be in limiting amounts for D1 receptors. Although the heterozygous mutant mice are apparently normal, their locomotor activity in response to the administration of several drugs of abuse (amphetamine, cocaine, morphine) is markedly diminished (Herve et al., 2001) (Corvol JC, Valjent E, Herve D, and Girault J-A, unpublished data). These effects are known to be dependent on D1 receptor-mediated signaling mainly at the level of the nucleus accumbens and their clear reduction in heterozygous mice shows that the $G\alpha_{olf}$ levels are determinant for the magnitude of acute behavioral responses to these drugs (Corvol et al., 2001). $G\alpha_{olf}$ levels are increased in the striatum following degeneration of dopamine neurons both in humans and rodents (Hervé et al. 1993; Corvol et al. 2001). These changes are likely to account for the functional hypersensitivity of D1 receptor observed in these conditions.

In transfection experiments, only minor differences have been detected between the $G\alpha_{olf}$ and the $G\alpha_s$ (Jones et al., 1990; Liu et al., 2001b). $G\alpha_{olf}$ displays a lower affinity for GDP and its stimulation factor on the adenylyl cyclase activity (stimulated to basal activity ratio) appears to be greater (Liu et al., 2001b). It is possible that $G\alpha_{olf}$ and $G\alpha_s$ also differ by the regulation of their gene expression. The expression of $G\alpha_{olf}$ appears to be rather complex, since at least four different transcripts are generated by utilization of two promoters, three polyadenylation sites and alternative splicing. Although all these mRNA species contain the same coding sequence, they are expressed in different ratios in the brain and in the olfactory epithelium and appear to have very different abilities to be translated (Herve et al., 1995). This could imply a yet unevaluated regulation of $G\alpha_{olf}$ in striatal areas.

The $\beta\gamma$ complexes associated with $G\alpha_{olf}$ in the striatum appear to contain the $G\gamma_7$ subunit. This subunit is highly expressed in the medium-sized spiny neurons containing D1 receptors (Watson et al., 1994) and in a strain of knockout mice, the loss of $G\gamma_7$ produces drastic reductions in the levels of $G\alpha_{olf}$ and in the D1 effects on cAMP production in the striatum (Schwindinger et al., 2003). Moreover, in transfected HEK 293 cells, $G\gamma_7$ is required for D1, but not D5 stimulation of cAMP production and appears to associate specifically with the $G\beta_1$ subunit (Wang et al., 1999, 2001). Interestingly the absence of $G\gamma_7$ does not alter the levels of $G\alpha_s$ in HEK 293 cells, whereas it reduces those of $G\alpha_{olf}$ in $G\gamma_7$ knockout mice, suggesting that $G\gamma_7$ stabilizes specifically $G\alpha_{olf}$ (Wang et al., 1999; Schwindinger et al., 2003). In conclusion, these studies show that D1 receptor interacts with a specific G protein composed of $G\alpha_{olf}$, $G\gamma_7$, and maybe $G\beta_1$, raising the intriguing question of the physiological consequences of such a G protein specialization.

3.1.2. cAMP production and degradation

Receptor-activated $G\alpha_{olf}$ stimulates cAMP synthesis by increasing the activity of adenylyl cyclase. Molecular cloning has led to the discovery of ten isotypes (ACI to ACX) of the

enzyme in mammals, having different mechanisms of activation (see review, Patel et al., 2001). Only four isoforms (ACII, ACV, ACVIII and ACIX) are expressed in the striatum at significant levels (Mons and Cooper, 1995). High levels of ACV mRNA are found homogeneously in the medium-sized spiny neurons suggesting that this isoform is more particularly involved in D1 receptor signaling (Glatt and Snyder, 1993; Mons and Cooper, 1994; Matsuoka et al., 1997). In agreement with this view, the deletion of the ACV gene in mice reduced the adenylyl cyclase activity stimulated by D1 agonist by more than 85%, but, surprisingly, it did not alter the behavioral responses to the administration of D1 agonists (Lee et al., 2002b; Iwamoto et al., 2003). Low levels of ACIX were also detected in medium-sized spiny neurons (Antoni et al., 1998) whereas ACII was predominantly expressed by large cholinergic neurons in the striatum (Mons and Cooper, 1995). The levels of ACVIII appeared to be low in the striatum. ACV is activated by $G\alpha_s$-type protein as all the other adenylyl cyclase isotypes (except ACX), but is characterized by its negative regulation by Ca^{2+}, by phosphorylation, and by $G\alpha_i$ subunits (Patel et al., 2001). Interestingly, ACIX is also inhibited by intracellular Ca^{2+} but through a mechanism involving calcineurin (Paterson et al., 2000).

Intracellular cAMP is degraded by phosphodiesterases: the most abundantly expressed isoform of this enzyme in the medium-sized spiny neurons is PDE1B (Polli and Kincaid, 1994). Mice lacking functional PDE1B display exaggerated behavioral and biochemical responses to D1 agonist stimulation, including increased PKA-dependent protein phosphorylation in striatal slices (Reed et al., 2002). PDE1B is a member of the phosphodiesterase family that is stimulated by the Ca^{2+}/calmodulin complex (Polli and Kincaid, 1992). Thus, intracellular Ca^{2+} can provide a potent inhibitory regulation on cAMP signaling in medium-sized spiny neurons of striatum since it is capable of inhibiting adenylyl cyclase (ACV) and stimulating phosphodiesterase (PDEB1) activities in these cells.

3.1.3. cAMP-dependent protein kinase

The role of cAMP produced by adenylyl cyclase is mainly to activate the cAMP-dependent protein kinase (PKA). In its inactive state, PKA is a tetrameric protein composed of two catalytic (C) subunits and two regulatory (R) subunits. The R subunits possess a binding site highly specific for cAMP and when cAMP binds the two PKA R subunits, the subunits of PKA dissociate, releasing fully active C subunits in the cytoplasm and the nucleus (Taylor et al., 1988). The C subunit transfers the γ phosphoryl group of ATP to the hydroxyl group of serine or threonine residues located in the consensus amino acid sequence (R/K_2-x-S/T). PKA has a relatively large spectrum of protein substrates, including ion channels, receptors and neurotransmitter-synthesizing enzymes as well as transcription factors when they diffuse into the nucleus (see below).

The C and R subunits are encoded by three (α, β, γ) and four (Iα, Iβ, IIα, IIβ) genes, respectively (Tasken et al., 1997). In striatal areas, Cα, Cβ and RIIβ are the predominantly expressed isoforms (Brandon et al., 1997). The mice deficient in functional RIIβ subunit show a decreased PKA activity in the striatum and exhibit behavioral modifications that suggest an alteration in their dopamine neurotransmission (Brandon et al., 1998). In neurons, the presence of RII targets the PKA towards the postsynaptic densities whereas PKA containing RI subunits are essentially localized in the cytoplasm (Corbin et al., 1977; Deviller et al., 1984). The association of RII subunits with these subcellular compartments is due to their interaction with A kinase attachment proteins (AKAP) (Carr et al., 1992;

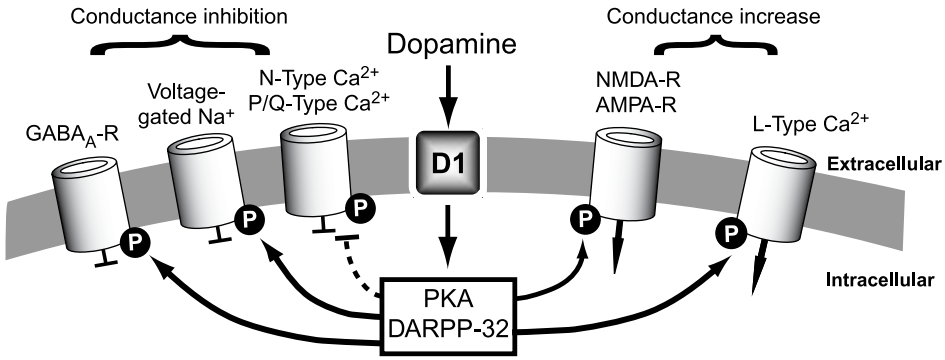

Fig. 2. Regulation of ion channels by D1/PKA/DARPP-32 in the striatum. Activation of D1 receptor regulates the PKA/DARPP-32 module and increases the phosphorylation state of voltage-gated Na^+ and L-type Ca^{2+} channels as well as $GABA_A$ receptor and NMDA- and AMPA-type glutamate receptors. The PKA/DARPP-32 module induces indirectly the dephosphorylation of N- and P/Q-type Ca^{2+} channels probably by promoting the targeting of PP-1 toward these channels. Phosphorylation has a positive effect on ionic conductance for glutamate receptors and Ca^{2+} channels whereas it displays a negative influence on ionic conductance for $GABA_A$ receptors and voltage-gated Na^+ channels (see text for references).

Coghlan et al., 1995). In the brain, members of the AKAP79/150 family are abundantly expressed and the murine protein AKAP150 was shown to be enriched in the medium-sized spiny neurons (Glantz et al., 1992; Ventra et al., 1996). AKAP79/150 are scaffolding proteins that bind, in addition to RII subunits, Protein Kinase C (PKC), calcineurin (PP-2B), β2-adrenergic receptor and members of MAGUK family of scaffolding proteins such as PSD-95 or SAP-97 which associate with many proteins in postsynaptic densities including glutamate receptors (Dodge and Scott, 2000). The role of AKAP appears thus to concentrate PKA at the synapse in close vicinity of receptors, ion channels and other signaling proteins. The formation of these multiunit complexes is supposed to increase the specificity, rapidity and efficiency of second messenger actions in neurons (see below).

3.2. D1-CONTROLLED REGULATION OF PROTEIN PHOSPHATASE 1

3.2.1. DARPP-32

A phosphoprotein termed DARPP-32 (Dopamine- and cAMP-regulated phosphoprotein, 32 kDa) plays a central role in the regulation of protein phosphorylation induced by D1 receptor stimulation in the striatum (Greengard et al., 1999). DARPP-32 is expressed at very high concentrations (about 50 μM) in all the medium-sized spiny neurons bearing D1 receptors (Ouimet et al., 1984, 1998). However, DARPP-32 is not specific of these neurons since it is detected in several populations of neuronal and nonneuronal cells expressing few or no D1 receptor, including striatopallidal neurons which express predominantly D2 receptors (Ouimet et al., 1998). Both in vivo and in vitro in brain slices, the activation of PKA by D1 receptor agonists was shown to increase the phosphorylation of Thr-34 residue in DARPP-32 (Walaas et al., 1983a). When phosphorylated at this site, DARPP-32 becomes a very potent inhibitor of protein phosphatase 1 (PP-1) with an $IC50 < 10^{-9}$ M and can amplify the effect of PKA activation (Hemmings et al., 1984b; Desdouits et al.,

1995c). DARPP-32 belongs to a group of proteins (which also includes inhibitor-1, and inhibitor-2) that is capable of inhibiting the catalytic subunit of PP-1, a highly conserved, broad spectrum protein phosphatase (Shenolikar and Nairn, 1991; Bollen, 2001; Cohen, 2002). Inhibitor-1 and DARPP-32 share a conserved domain that inhibits PP-1 only when phosphorylated on a critical threonine residue (Thr-34 in DARPP-32, Thr-35 in inhibitor-1), whereas inhibitor-2 is active in its unphosphorylated form (see Bollen, 2001; Cohen, 2002). Although in lower amounts than DARPP-32, inhibitor-1 is present in striatal neurons and could participate in the D1 receptor signaling (Nairn et al., 1988; Hemmings et al., 1992). The dephosphorylation of Thr-34 in DARPP-32 can be performed by two protein phosphatases, PP-2A and PP-2B, also known as calcineurin (King et al., 1984; Hemmings et al., 1990). Calcineurin which is abundantly expressed in the striatal neurons is activated by the Ca^{2+}/calmodulin complex. When intracellular Ca^{2+} elevations are produced by NMDA stimulation in the striatum (Halpain et al., 1990) or by depolarization in striatonigral terminals, calcineurin dephosphorylates the Thr-34 residue of DARPP-32 (Desdouits F, Siciliano JC, and Girault J-A, unpublished observations). These effects constitute additional examples of the inhibitory action of calcium on D1 receptor signal transduction in the striatum. In vitro, the Thr-34 of DARPP-32 is also phosphorylated by cGMP-dependent protein kinase (PKG) with a high efficacy (Hemmings et al., 1984a). In slices of substantia nigra the selective stimulation of PKG by nitric oxide-induced activation of cGMP production increases the phosphorylation of DARPP-32 at Thr-34 (Tsou et al., 1993).

Besides Thr-34 residue, DARPP-32 is also phosphorylated at other serine and threonine residues by various protein kinases and these phosphorylations have important regulatory effects. In resting striatal neurons, DARPP-32 is phosphorylated at Thr-75 site by cyclin-dependent kinase 5 (CDK5) and in this phosphorylated state, DARPP-32 becomes a potent inhibitor of PKA (Bibb et al., 1999). When D1 receptors are stimulated in vivo or in striatal slices, Thr-75 is dephosphorylated through activation of protein phosphatase 2A (PP-2A), that removes the inhibitory constraint exerted on PKA (Nishi et al., 2000). DARPP-32 appears thus to be an important D1 receptor-triggered switch regulating cAMP-dependent protein phosphorylation in the striatum: when D1 receptor is not stimulated, DARPP-32 blocks PKA, whereas following D1 receptor stimulation, it enables PKA signaling by inhibiting PP-1. Interestingly, CDK5 expression is increased by Δ-FosB, a transcription factor induced by chronic treatment by cocaine and this control may participate in the neuronal changes responsible for cocaine addiction (Bibb et al., 2001).

DARPP-32 is also phosphorylated on Ser-137 and on Ser-102 (and perhaps Ser-45) by casein kinase 1 and 2 (CK1 and CK2), respectively (Girault et al., 1989, 1990b; Desdouits et al., 1995b). The phosphorylation of DARPP-32 on these Ser residues has no influence on the activity of PKA or PP-1. However, by different mechanisms, phosphorylation by CK1 and CK2 enhances the phosphorylation of DARPP-32 on Thr-34 residue (Girault et al., 1989; Desdouits et al., 1995a) and therefore increases the PKA/DARPP-32-dependent signaling stimulated by D1 receptors.

Interestingly, glutamate is able to affect the phosphorylation of DARPP-32 at these various sites. In addition to decreasing Thr-34 phosphorylation, the stimulation of NMDA and AMPA receptors was shown to decrease Thr-75 phosphorylation through a Ca^{2+}-dependent activation of PP-2A (Nishi et al., 2002). Moreover, in striatal slices, the stimulation of type-I metabotropic glutamate receptor increased the phosphorylation on Thr-34, Thr-75 and Ser-137 by various mechanisms depending on CDK5, CK2 or Extracellular signal-Regulated Kinase (ERK) (Liu et al., 2001a; Nishi et al., 2003). These

results provide evidence for a complex effect of glutamate on D1 receptor signaling at the level of DARPP-32 regulation.

3.2.2. Protein Phosphatase 1

Because PP-1 is inhibited by phospho-DARPP-32, this phosphatase appears central for the intracellular events triggered by activation of D1 receptor. PP-1 is a ubiquitously expressed enzyme having the ability to dephosphorylate Ser and Thr residues in a broad variety of cellular proteins. The native structure of PP-1 is a 1 : 1 complex composed of a catalytic subunit that differs little from one isoform to another, and a number of different inhibitory or targeting proteins which determine to a large extent, the diversity of PP-1 functions (see reviews, Shenolikar and Nairn, 1991; Wera and Hemmings, 1995; Bollen, 2001; Cohen, 2002). Many regulatory proteins contain a common motif (R/K)(V/I) × F that is responsible for the interaction with catalytic PP-1 subunit and that explains why the binding of various regulatory proteins are mutually exclusive (Egloff et al., 1997). DARPP-32 contains such a docking domain and the PP-1 inhibition by phospho-DARPP-32 results from a double interaction, involving both the docking domain, and phospho-Thr34 and the surrounding residues that occupy or bind close to the active site of PP-1 (Desdouits et al., 1995c; Kwon et al., 1997; Huang et al., 1999).

DARPP-32 and inhibitors 1 and 2 correspond to inhibitory proteins interacting with free catalytic subunits. A distinct group of proteins serves to localize PP-1 in restricted cellular compartments. In dopaminoceptive neurons, PP-1 is highly enriched in dendritic spines and in postsynaptic densities (Ouimet et al., 1995), and this is due to its interaction with two homologous proteins, spinophilin and neurabin (neuronal actin binding protein) (Allen et al., 1997; Nakanishi et al., 1997; McAvoy et al., 1999). Both proteins contain a PDZ domain, which has the potential of binding synaptic proteins including ion channels and receptors. Interestingly, both proteins were shown to interact with F-actin, raising the possibility that these proteins bring PP-1 into the vicinity of the dense actin network present in dendritic spines and may contribute to the control of spine morphology and the synaptic plasticity (Oliver et al., 2002). PKA stimulation or activation of D1 receptors produce the phosphorylation of spinophilin in the domain of interaction with F-actin, inducing a loss of association (Hsieh-Wilson et al., 2003). The PKA pathway could thus modulate the anchoring of spinophilin in dendritic spine and, because of the interaction of spinophilin with PP-1 and AMPA glutamate receptors, could control to the efficacy and the plasticity of synaptic transmission.

3.3. TARGET PROTEINS FOR D1 RECEPTOR-REGULATED cAMP PATHWAY

3.3.1. cAMP-dependent phosphoproteins in the striatum

In an effort to identify the protein substrates for PKA following activation of D1 receptor in striatal neurons, the Greengard's lab has characterized several phosphoproteins and has defined the functions for some of them including DARPP-32 (Walaas et al., 1983b, c). One of those proteins, ARPP-16, is highly enriched in the striatal areas and is phosphorylated by PKA following stimulation by D1 receptor agonist in striatal slices (Girault et al., 1990a). This protein is close to a ubiquitously expressed phosphoprotein ARPP-19 (Girault et al., 1990c; Horiuchi et al., 1990; Dulubova et al., 2001) that has recently been shown in PC12 cells to bind the GAP43 mRNAs and to regulate their stability via a

PKA-dependent mechanism (Irwin et al., 2002). ARPP-21 is also a PKA substrate which is enriched in dopaminoceptive neurons and in cortical neurons (Hemmings et al., 1989; Girault et al., 1990a; Caporaso et al., 2000). ARPP-21 is a regulator of Ca^{2+}/calmodulin signaling controlled by phosphorylation (Rakhilin et al., 2004). In the late 1980s and in 1990s, the intense activity of molecular cloning has allowed the identification of numerous consensus sites for PKA in the amino acid sequence of proteins important for neuron functions, raising the possibility that their activity could be regulated by D1 receptor-dependent cAMP pathway.

3.3.2. Sodium channels

In neurons acutely dissociated from the striatum, cortex and hippocampus, the stimulation of a D1-like receptor decreases voltage-gated Na^+ currents, affecting the amplitude of peak currents without any significant change in the kinetics or voltage-dependence of activation or inactivation (Surmeier et al., 1992; Schiffmann et al., 1995; Cantrell et al., 1997; Maurice et al., 2001). This effect is mediated through activation of PKA, since it is mimicked by intracellular application of PKA catalytic subunit and antagonized by PKA inhibitor (PKI) (Schiffmann et al., 1995). The brain-expressed Na^+ channels contain an α subunit, Nav 1.2 that possesses five potential phosphorylation sites for PKA in an intracellular regulatory loop (Rossie et al., 1987; Cantrell and Catterall, 2001) and studies in *Xenopus* oocytes and in transfected mammalian cells have shown that the PKA-dependent phosphorylation of Ser573 in this Na^+ channel subunit reproduces largely the effects observed in neurons (Cantrell et al., 1997; Smith and Goldin, 1997). In addition, dopamine suppresses essentially the rapidly inactivating Na^+ channels in cortical neurons that express Nav1.1 or Nav1.2 subunits (Maurice et al., 2001). In striatal neurons, the modulation of Na^+ channels by dopamine is regulated by phosphorylation of DARPP-32 and inhibition of PP-1 (Schiffmann et al., 1998). Interestingly, AKAP15 that associates Nav1.2 subunits and RIIα regulatory subunits of PKA, is required for the development of dopamine effect on Na^+ currents in hippocampal neurons (Cantrell et al., 1999; Cantrell et al., 2002). The efficiency of regulation by dopamine is increased when the neurons are depolarized or following PKC stimulation, suggesting complex cross-talk between PKA- and PKC-dependent signaling at the levels of Na^+ channels (Cantrell et al., 2002).

3.3.3. Calcium channels

Striatal medium spiny neurons express L, N, P, Q, T and R-type Ca^{2+} channels (Bargas et al., 1994; Churchill and Macvicar, 1998). D1 receptor stimulation increases voltage-dependent L-type Ca^{2+} currents (Surmeier et al., 1995) by a mechanism that is essentially the same as that demonstrated in cardiac myocyte and that results from a PKA-dependent phosphorylation of α_1 and/or β subunits of the channels (Kamp and Hell, 2000). This upregulation of L-type Ca^{2+} channel appears to play a major contribution in the ability of dopamine to facilitate NMDA effects in striatal neurons (Cepeda et al., 1998).

In contrast, N- and P/Q-type Ca^{2+} currents are suppressed by stimulation of D1 receptors (Surmeier et al., 1995; Zhang et al., 2002). Surprisingly, inhibition of both PKA and PP-1 impairs the development of this effect. This suggests that the D1 receptor-dependent activation of PKA stimulates a PP-1-dependent dephosphorylation of Ca^{2+} channels, presumably by retargeting PP-1 in close vicinity of Ca^{2+} channels (Surmeier et al., 1995). In adrenal cells, dopamine inhibits T-type Ca^{2+} channel by stimulating

D1-like receptors and this effect requires a combined action of Gβγ and PKA activation (Drolet et al., 1997).

3.3.4. AMPA-type glutamate receptors

In vivo treatments and studies on striatal slices have shown that the activation of D1 receptors phosphorylates the GluR1 subunit of AMPA receptors on Ser845 by activating PKA and by phosphorylating DARPP-32 (Snyder et al., 2000). Phosphorylation of GluR1 at Ser845 increases the channel open time (Banke et al., 2000) and also constitutes a critical factor regulating GluR1 receptor trafficking towards the synaptic membrane and synaptic plasticity in the hippocampus (Esteban et al., 2003; Lee et al., 2003). In agreement with this view, phosphorylation of GluR1 was found to correlate with changes in the synaptic strength in the hippocampus (Lee et al., 2000). In striatal neurons, electrophysiological recordings provide conflicting results concerning the effect of D1 receptor stimulation on AMPA currents, suggesting a complex interplay between various ionic conductances regulated by dopamine (see review, Nicola et al., 2000). However, several studies have reported that AMPA-stimulated currents are increased by D1 receptor stimulation (Galarraga et al., 1997; Umemiya and Raymond, 1997; Yan et al., 1999b). Association of PKA with AKAP79 and spinophilin has been shown to regulate the phosphorylation of GluR1 at Ser845 by recruiting calcineurin and PP-1 respectively (Yan et al., 1999b; Tavalin et al., 2002). In the striatum, phosphorylation of DARPP-32 by PKA amplifies the GluR1 phosphorylation by sequestrating and inhibiting the PP-1 associated with GluR1 and spinophilin (Yan et al., 1999b).

3.3.5. NMDA-type glutamate receptors

The NR1 subunit, the presence of which is required to form functional NMDA-type receptors, was found to be phosphorylated by dopamine in striatal slices (Snyder et al., 1998). This effect was mimicked by a D1 receptor selective agonist and an activator of cAMP production, whereas it was blocked by a specific inhibitor of protein kinase A (Snyder et al., 1998). This effect was abolished in the striatum of mice with deleted DARPP-32 gene, suggesting the implication of a PKA/DARPP-32/PP-1 cascade (Fienberg et al., 1998; Flores-Hernandez et al., 2002). Experiments using *Xenopus* oocytes injected with striatal mRNAs have supported the role of these intracellular cascades in the increase in NMDA receptor currents (Blank et al., 1997). In striatal slices, they could also contribute to the increase of electrophysiological responses to NMDA produced by D1 receptor stimulation (Cepeda et al., 1998; Flores-Hernandez et al., 2002). These effects do not exclude other effects due to the direct interaction of D1 receptor with NR1 and NR2B subunits (Lee et al. 2002a).

3.3.6. $GABA_A$ receptors

Application of a D1 receptor agonist reduces GABA-evoked currents in neurons from the striatum or olfactory bulb (Brunig et al., 1999; Flores-Hernandez et al., 2000). This effect, in striatal medium spiny neurons, is mediated through the activation of PKA/DARPP-32/PP-1 cascade, since it is blocked by an inhibitor of PKA or by the absence of DARPP-32 in mutant mice while it is mimicked by inhibitor of PP-1 (Flores-Hernandez et al., 2000). Stimulation of the D1 receptor induces a phosphorylation of β2/β3 $GABA_A$

receptor subunits, an effect significantly attenuated in mice bearing a null mutation of the DARPP-32 gene. In striatal cholinergic interneurons, the D5 receptors are more abundantly expressed than the D1 receptors. Their stimulation up-regulates a subset of $GABA_A$ receptor sensitive to Zn^{2+} ion (Yan and Surmeier, 1997). Although dependent upon PKA activation, the modulation was blocked by PP-1 inhibition suggesting that it resulted from the dephosphorylation of a particular subset of $GABA_A$ receptors. It is unlikely that the regulation was due to the direct interaction of D5 receptor with $GABA_A$ receptor containing $\gamma 2$ subunit, since the interaction leads to a mutual inhibition of receptors (Liu et al., 2000).

3.3.7. Na^+/K^+-dependent ATPase

One target of D1 receptor activation is the electrogenic ion pump Na^+/K^+-dependent ATPase, which is crucial for the generation and maintenance of ion gradients and membrane potential in neurons. Dopamine, in part through an effect on D1 receptor, reduced the activity of Na^+/K^+-dependent ATPase in isolated striatal neurons as well as in renal tubules (Bertorello et al., 1990; Aperia et al., 1991). In striatal neurons, dopamine affects specific isoforms of the enzyme (Nishi et al., 1999). However, the mechanism of this effect remains unclear since Na^+/K^+ ATPase isoforms are apparently not directly phosphorylated (Nishi et al., 1999). However, the D1 receptor/PKA/DARPP-32 cascade is involved since the ability of dopamine to inhibit Na^+/K^+-dependent ATPase is impaired in striatal neurons lacking DARPP-32 (Fienberg et al., 1998).

3.3.8. Modulation of excitability of striatal neurons by D1 receptor

Activation of D1 dopamine receptor results in divergent effects on a wide array of ionic conductances, including inhibitory influences on voltage-gated Na^+ channels and N or P/Q-type Ca^{2+} channels, as well as stimulatory influences on L-type Ca^{2+} channels and glutamate receptors of NMDA and AMPA types. It has been proposed that the overall electrophysiological effects of D1 receptor activation depend on the depolarized or hyperpolarized states of membrane potentials (Wilson and Kawaguchi, 1996; Hernandez-Lopez et al., 1997). Striatal neurons have been shown to oscillate between two preferred levels of membrane potential (approximately, -85 mV and -55 mV), depending on the activity in convergent excitatory inputs (Wilson and Kawaguchi, 1996). When striatal neurons are in a depolarized state, D1 activation increases the neuronal excitability, presumably by modulating L-type Ca^{2+} channels, glutamate receptors and $GABA_A$ receptors (Wilson and Kawaguchi, 1996; Cepeda et al., 1998). By contrast, when neurons are in a hyperpolarized state, D1 activation appears to reduce the evoked excitations, probably by inhibiting N- and P/Q- type Ca^{2+} channels and by enhancing inward rectifying K^+ channels (see review, Nicola et al., 2000). Thus, the activation of D1 receptors appears to produce opposite effects, depending on the membrane potential. This may enhance the contrast between the two states of membrane potential and augments the signal/noise ratio in the activity of striatal neurons.

Numerous studies have demonstrated synaptic plasticity at the levels of corticostriatal glutamate synapses, exhibiting either LTD or LTP depending on the experimental conditions (see reviews, Centonze et al., 2001; Reynolds and Wickens, 2002). D1-type receptors and DARPP-32 are required for induction of both LTD and LTP (Calabresi et al., 2000; Kerr and Wickens, 2001). In the hippocampus and prefrontal cortex,

dopamine also regulates LTP and LTD by acting through D1 receptors (Huang and Kandel, 1995; Chen et al., 1996; Matthies et al., 1997; Otmakhova and Lisman 1998; Gurden et al., 2000), and several components of the intracellular signaling related to D1 receptor-activation have been identified to be the important effectors of LTP and LTD in the hippocampus, including PP-1, spinophilin, GluR1 subunit of AMPA receptor and CREB (cAMP-responsive element binding protein) (Bourtchuladze et al., 1994; Allen et al., 2000; Morishita et al., 2001; Lee et al., 2003). These studies open the possibility for additional intracellular mechanisms involved in the D1 receptor-regulated synaptic plasticity in the striatum.

3.4. ALTERNATIVE SIGNAL TRANSDUCTION OF D1 RECEPTORS

3.4.1. Possible coupling of D1-type receptors with Gi/o and Gq protein

In reconstitution experiments with solubilized proteins, D1 receptors have been shown to interact not only with $G\alpha_s$ but also with pertussis toxin (PTX)-sensitive α subunits of G protein (Sidhu et al., 1991). In cell lines expressing D1 receptors, solubilized D1 receptors can be co-immunoprecipitated by antisera directed against $G\alpha_s$ or $G\alpha_o$ suggesting an alternative coupling of D1 receptor with Go protein (Kimura et al., 1995). By contrast, D5 receptor does not co-immunoprecipate with PTX-sensitive α subunits but with $G\alpha_z$ a protein of the $G\alpha_{i/o}$ type which is insensitive to PTX (Sidhu et al., 1998).

In various brain areas and in kidney, D1 agonists were found to stimulate phospholipase C (PLC) activity (Undie and Friedman, 1990; Undie et al., 1994). In the hippocampus, the amygdala, the cortex and the striatum, anti-$G\alpha_q$ antibodies co-immunoprecipitate receptors able to bind the specific D1 antagonist SCH23390 (Friedman et al., 1997; Jin et al., 2001). However, these receptors do not appear to be bona fide D1 receptors since the D1-type responses on PLC activity appear normal in D1 receptor knockout mice (Friedman et al., 1997). They do not seem to correspond either to D5 receptors since these latter receptors are not coupled to $G\alpha_q$ and are unable to mediate PLC responses (Sidhu et al., 1998). It should also be noted that the PLC responses are obtained with high concentrations (100 μM) of the D1 agonist SKF38393 and that, in several cell lines transfected with the D1 receptor cDNA stimulation of D1 receptor has no effect on the PLC activity (Dearry et al., 1990; Tiberi et al., 1991; Pedersen et al., 1994; Sugamori et al., 1994).

3.4.2. Protein–protein interactions of D1-type receptors

It has been reported that D1 receptor stimulation can induce an intracellular mobilization of Ca^{2+} via the interaction of D1 receptor with a novel protein, calcyon (Lezcano et al., 2000). By a two-hybrid screening in yeast, calcyon was shown to associate with the C-terminal tail of D1 and D5 receptor. Activation of PKC by various stimulants allowed calcyon to couple the D1 or possibly D5 receptors with Gq protein, rendering these receptors able to produce intracellular Ca^{2+} mobilization. Calcyon is expressed at higher levels in the cortex than in the striatum and the intracellular calcium release induced by D1/D5 receptor stimulation is detected in cortical and hippocampal neurons in culture, but not in striatal neurons (Lezcano and Bergson, 2002; Zelenin et al., 2002).

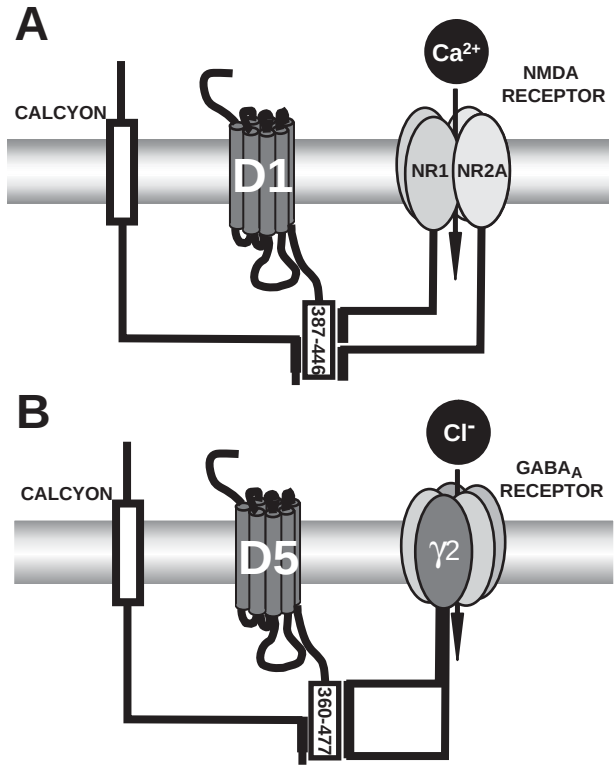

Fig. 3. Interaction of D1-type receptors with proteins other than G proteins. Recent work suggests that D1-type receptors interact directly with several proteins, including ionotropic receptors, and thus alter signal transduction or receptor properties. The C-terminal region of D1 (A) and D5 (B) interacts with non G proteins. Both receptors are able to associate with the transmembrane protein, calcyon. Calcyon enables D1 and possibly D5 receptors to stimulate intracellular Ca^{2+} release. This signaling requires a previous activation of PKC but the precise mechanism for this priming is unknown. The C-terminal tail of D1 receptor displays two domains able to interact with the NR1 and NR2A subunits of NMDA-type glutamate receptors, respectively. When activated, D1 receptor inhibits NMDA-triggered currents through its interaction with NR2A subunit whereas it initiates an anti-apoptotic signal via its association with NR1. The C-terminal tail of D5 receptor associates with the γ2 subunit of $GABA_A$ receptors producing crossed inhibition of both receptors (see text for references).

In a recent report, D1 receptors were shown to modulate NMDA receptor-mediated glutamate functions through direct protein–protein interactions (Lee et al., 2002a). The c-terminal tail of D1 receptors displays two distinct domains of interaction with the NR1 and NR2A subunits of the NMDA-type receptor. Activation of D1 receptor inhibits NMDA-triggered currents through the interaction of D1 receptor with NR2A subunit without the intervention of any G protein. Agonist-stimulation of D1 receptors results in their dissociation from NR1, allowing the initiation of an anti-apoptotic signal through the recruitment of calmodulin and PI-3-kinase by NMDA receptor. Through these mechanisms, activation of D1 receptor could reduce NMDA receptor-mediated excitotoxicity. In addition, association of D1 receptor with NMDA receptor may have important consequences for its intracellular trafficking (Fiorentini et al., 2003).

The C-terminal region of the D5 receptor did not interact with either NR1 or NR2A subunits of NMDA receptors, but was able to associate with the γ2 subunit of $GABA_A$

receptors (Liu et al., 2000). This association produced reciprocal cross-inhibitions of both receptors when they were stimulated by their specific agonists (Liu et al., 2000). These studies underline that a collection of proteins interacting directly with the D1-type receptors could modulate the G protein-mediated signaling as well as produce novel responses independent of the G protein.

3.4.3. Novel intracellular signaling triggered by D1-type receptor stimulation

In vivo treatments with addictive drugs that have the capacity to induce dopamine release in striatal areas have been shown to activate ERK in these brain regions (Valjent et al., 2000, 2001; Choe et al., 2002; Brunzell et al., 2003). These effects were blocked by pretreatments with the D1 receptor antagonist, SCH23390. In a culture of striatal cells, the D1 agonist SKF38393 also activated ERK and MEK, the specific kinase upstream of ERK (Vincent et al., 1998; Brami-Cherrier et al., 2002). Interestingly, the in vivo inhibition of MEK completely prevented behavioral conditioning triggered by chronic treatments of cocaine and tetrahydrocannabinol (conditioned place preference), but affected to a lesser extent the acute behavioral response (locomotor activity) (Valjent et al., 2000, 2001). These results suggest that the ERK signaling is mainly involved in the long-term effects of drugs on behavior rather than on the immediate responses to drugs.

Other signaling pathways involving mitogen-activated protein kinases (MAPKs) could be activated by D1 receptor stimulation, since in a human neuroblastoma cell line, the D1 agonist SKF38393 activates p38 MAPK and JNK by a PKA-dependent mechanism (Zhen et al., 1998). In addition, SKF38393 activates Akt in striatal neurons in culture by producing an increase in the phosphorylation levels of Akt at Thr308 residue (Brami-Cherrier et al., 2002). The intracellular events responsible for this effect are independent of PI3-kinase activity, but depend on the ERK pathway. The MAPK and Akt-dependent signaling pathways could transduce dopamine signals to the regulation of gene expression, especially by modulating CREB in striatal neurons (see below).

3.5. CONCLUSION

The intracellular events triggered by the activation of D1 receptor have been extensively studied in the medium spiny neurons of the striatum. In these neurons, the cAMP-controlled signal transduction plays a critical role, and involves specific isoforms of signaling proteins: $G\alpha_{olf}$ and $G\gamma_7$, ACV, PKA RIIβ, PDEB1 and DARPP-32. This reveals that the cAMP pathway is highly differentiated in striatal neurons, and provides some particularities to the regulation of this signaling pathway. In particular, we have mentioned that several elements are controlled by Ca^{2+}, which has a globally negative influence on the cAMP pathway in the striatum. The cascade constituted by the trio PKA/DARPP-32/PP-1 also confers an important individuality to the striatal cAMP pathway and exerts crucial regulatory influences on the functions of several ion channels and receptors, and, thereby, on the activity of neurons. In the last few years of research, it appeared that the D1 receptor can also act independently of the G protein by direct interactions with effector proteins and that the activation of the D1 receptor activates of the ERK and the Akt pathway. The relationships between the cAMP pathway and these novel signal transduction pathways, as well as their respective roles in dopamine-controlled responses remain to be precisely evaluated.

4. SIGNAL TRANSDUCTION OF D2-TYPE RECEPTORS

4.1. D2 RECEPTORS

4.1.1. Coupling with G proteins

In spite of the diversity of intracellular events produced by their stimulation, the D2 receptors are essentially coupled by Gi/o-type proteins to their effectors, since most cellular effects are abolished by PTX. Besides the sensory G proteins, transducins and gustducin expressed in the retina and tongue, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_o$ are the only G protein subunits which can be inhibited by PTX-dependent ADP-ribosylation. D2 receptors appear to have the highest affinity and efficacy of stimulation for $G\alpha_{i2}$ when reconstituted in phospholipid vesicles (Senogles et al., 1990). However, numerous studies show that the D2 receptor can activate second messenger pathways via the four $G\alpha_{i/o}$ proteins and their associated $\beta\gamma$ (see below). Depending on the G proteins and the effectors present in the various cells, as well as on their levels of expression, D2 receptors are susceptible to generate divergent signaling events. In the brain, most D2 receptors appear to be associated with $G\alpha_o$. Since GTP loses its ability to regulate the agonist affinity for D2 receptor in $G\alpha_o$-deficient mice, revealing an impaired coupling of D2 receptors with G protein (Jiang et al., 2001). By contrast, in mice deficient in $G\alpha_{i1}$ and $G\alpha_{i2}$ or in $G\alpha_{i1}$ and $G\alpha_{i2}$, GTP displayed normal effects (Jiang et al., 2001). However, since $G\alpha_o$ is by far the most abundant $G\alpha$ protein, reaching up to 1% of membrane proteins in brain, it is possible that the lack of $G\alpha_o$ was not compensated by the other $G\alpha$ proteins, whereas the consequences of the absence of the less abundant $G\alpha$ subunits could have been overlooked. These experiments do not rule out that D2 receptors also act through the Gi-type proteins in normal conditions.

4.1.2. Effects on adenylyl cyclase

Early in the 1980s, D2 receptor was found to have the ability to inhibit the activity of adenylyl cyclase. This effect was first discovered in the pituitary gland (De Camilli et al., 1979; Enjalbert and Bockaert, 1983) and then in adult and embryonic neurons of caudate putamen in the CNS (Onali et al., 1985; Weiss et al., 1985). The cloning of the D2 receptor has confirmed that this receptor subtype was responsible for the inhibition of the adenylyl cyclase (Albert et al., 1990). The ability of D2 receptors to inhibit cAMP production was found in most cell types, in which the receptor cDNA was transfected, making this effect, the most constant property of D2 receptor signaling. The only exceptions are cells expressing the adenylyl cyclase isoform ACII, the activity of which is insensitive to $G\alpha_i$ proteins, but is stimulated by $G\beta\gamma$, resulting in an activation of cAMP production by D2 agonists (Watts and Neve, 1997; Yao et al., 2002). The D2-mediated adenylyl cyclase inhibition is invariably abolished by treatment with PTX. The use of mutant G protein α subunits resistant to PTX led to the comparison of the roles of the various subunits in the adenylyl cyclase inhibition (Taussig et al., 1992). The results of these studies vary to some extent depending on the cellular models, but most conclude that $G\alpha_{i2}$ and $G\alpha_{i3}$ are the most efficient for transducing the D2 receptor-mediated inhibition of cAMP production (Senogles et al., 1990; Senogles, 1994; Guiramand et al., 1995; O'Hara et al., 1996; Ghahremani et al., 1999). The results for $G\alpha_{i1}$ and $G\alpha_o$ are more inconstant, the potency being low ($G\alpha_{i1}$)

or restricted to the long isoform of the D2 receptor ($G\alpha_o$) (Senogles et al., 1990; Senogles, 1994; Watts et al., 1998). In the pituitary tumor cells expressing D2 receptors, their coupling to adenylyl cyclase was blocked by antibodies inhibiting both $G\alpha_{i1}$ and $G\alpha_{i2}$, but not with those blocking $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_s$ or $G\alpha_q$ (Izenwasser and Cote, 1995). Other proteins could regulate the signal transduction of D2 receptors providing possible explanation for divergent results obtained in various cell models. Actin-Binding Protein 280 (ABP280) has been shown to interact with the third intracellular loop of D2S, D2L and D3 receptors. This interaction was found to potentiate adenylyl cyclase inhibition and membrane clustering of D2 receptors and to be regulated by phorbol esters (Li et al., 2000).

4.1.3. Effects on potassium channels

In pituitary adenoma cells, dopamine was found to activate K^+ currents through D2 type receptor, leading to cell hyperpolarization (Israel et al., 1985). Similar effects have been described in lactotroph and melanotroph cells in the anterior pituitary as well as in the mesencephalic neurons in the CNS (Israel et al., 1985; Lacey et al., 1988; Greif et al., 1995). The K^+ current-induced hyperpolarization appears to underlie the inhibition of DA release mediated by D2 autoreceptors in dopamine neurons and of prolactin release in lactotroph cells. In particular, the blockade of K^+ channel by 4-aminopyridine or tetramethylammonium abolished the inhibitory effect of D2 agonists on DA release (Bowyer and Weiner, 1989; Cass and Zahniser, 1991; Tang et al., 1994a).

The activation of K^+ channels by D2 receptors involves Gi/o proteins since it was abolished by PTX (Lledo et al., 1990; Einhorn and Oxford, 1993). In pituitary cells, the effect is independent of the D2-dependent inhibition of adenylyl cyclase since drugs blocking or stimulating cAMP pathway are ineffective for regulating K^+ channels (Lledo et al., 1990; Einhorn and Oxford, 1993). The regulated K^+ conductance has inward-rectifying properties and it is likely that D2 receptor could affect G protein-regulated Inward Rectifier K^+ channels (GIRK) via G protein $\beta\gamma$ subunits. D2 receptors have been shown to activate heteromeric GIRKs associating Kir3.1 and Kir3.4 in lactotrophs as well as homomeric GIRKs containing only Kir3.2 subunits like those expressed by dopamine neurons in the substantia nigra (Inanobe et al., 1999; Kuzhikandathil and Oxford, 2000; Gregerson et al., 2001). The $G\beta\gamma$ subunits activating K^+ channels could be provided by $G\alpha_{i3}$ in lactrophs or $G\alpha_o$ in dopamine neurons since D2 receptor effects are blocked by antibodies against $G\alpha_{i3}$ and $G\alpha_o$ when infused into the two types of cells respectively (Lledo et al., 1992; Liu et al., 1999).

In striatal neurons, the effects of D2 receptor activation on K^+ channels are more complex. Stimulation of the D2 receptor has been reported to open a K^+ channel that displays a 85 pS conductance and a weak inward rectification, and this rectification seems to differ from that found in pituitary cells (Freedman and Weight, 1988; Einhorn et al., 1991; Greif et al., 1995). In contrast, D2 receptor activation was also reported to suppress K^+ currents probably through Kir2 channels (Uchimura and North, 1990). However, the activation of K^+ channels occurs in a membrane-delimited manner via $G\beta\gamma$ mobilization, whereas inhibition of K^+ channels could result from the D2 receptor mediated inhibition of adenylyl cyclase and the dephosphorylation of Kir2 subunit at its PKA sensitive site (Nicola et al., 2000).

4.1.4. Effects on intracellular calcium

In pituitary- or neuron-derived cells, the activation of D2 receptor induces the closing of voltage-dependent Ca^{2+} channels via a cAMP-independent mechanism (Vallar et al., 1990). The hyperpolarization induced by the activation of K^+ currents could contribute to this effect (Vallar et al., 1990), but it appeared to result more probably from direct effects on Ca^{2+} channels. In lactotrophs, the intracellular injection of antibodies recognizing $G\alpha_o$ blocks the action of D2 receptor on K^+ currents without affecting Ca^{2+} currents, whereas the effects on the Ca^{2+} current are specifically blocked by anti-$G\alpha_{i3}$ antibodies (Lledo et al., 1992). In striatal cholinergic interneurons, D2 agonists inhibit N-type calcium channels without the intervention of an intracellular second messenger, suggesting a direct action of $G\beta\gamma$ subunits on Ca^{2+} channels (Yan et al., 1997).

However, in many other cell types, activation of the D2 receptor was found to increase the concentration of cytosolic free Ca^{2+}, by the activation of inositol triphosphate production and mobilization of Ca^{2+} from intracellular stores. This effect was observed when D2 receptors were transfected in fibroblastic cell lines, but not in cell lines derived from pituitary or from dopamine neurons (Vallar et al., 1990; Tang et al., 1994b). This latter observation is in agreement with results showing that D2 agonists have no action on Ca^{2+} currents, or may even inhibit PLC activity in pituitary cells (Journot et al., 1987; Vallar et al., 1990; Rasolonjanahary et al., 2002). Following transfection in appropriate cells, D2 receptor is capable of mobilizing Ca^{2+} via a mechanism sensitive to PTX, but independent from cAMP. When $G\beta\gamma$ activity was antagonized by the carboxy-terminal domain of G protein receptor kinase, the D2-induced Ca^{2+} mobilization was blocked in 3T3 fibroblastic cells, suggesting that this effect resulted from the activation by $G\beta\gamma$ of $PLC\beta_{2/3}$ present in these cells (Ghahremani et al., 1999). Like in the endocrine cells, a membrane hyperpolarization was observed in fibroblastic cells, but it resulted from the activation of Ca^{2+}-dependent K^+ channels (Vallar et al., 1990).

Early studies reported the lack of stimulation of PLC activity by D2 agonist in the striatum but the measures were taken in striatal slices in which D2 agonists could have very complex effects (Kelly et al., 1988; Pizzi et al., 1988; Rubinstein and Hitzemann, 1990). Mobilization of Ca^{2+} from intracellular stores by D2 receptor activation has been observed in striatal medium spiny neurons (Hernandez-Lopez et al., 2000). The medium spiny neurons express PLCβ1 and the D2 receptor activation could stimulate this enzyme by mobilizing $G\beta\gamma$ subunits (Hernandez-Lopez et al., 2000). The elevation of intracellular Ca^{2+} in medium spiny neurons was very transient, since it promoted an immediate closure of L type-Ca^{2+} channels, due to the activation of the Ca^{2+}-dependent protein phosphatase, calcineurin and subsequent dephosphorylation of the L-type Ca^{2+} channels (Hernandez-Lopez et al., 2000).

4.1.5. D2 receptor-mediated protein phosphorylation

Several studies have revealed that the D2 receptors modulate the state of phosphorylation of DARPP-32. In mouse striatal slices, the D2 agonist quinpirole decreased the basal phosphorylation of DARPP-32 on Thr-34, and antagonized the phosphorylation produced by the application of D1 agonist, forskolin or 8-bromo-cAMP (Nishi et al., 1997). This D2 effect was calcium-dependent and was blocked by cyclosporine A, an inhibitor of calcineurin, suggesting that it involved an increase in intracellular Ca^{2+} and a dephosphorylation of DARPP-32 by calcineurin. This study showed that an activation of

D1 and D2 receptors in the striatum exerts opposite effects on the state of phosphorylation of DARPP-32. Studies carried out in vivo have confirmed the importance of D2 receptors in regulating the state of phosphorylation of DARPP-32 since treatments with the D2 antagonist eticlopride produced an increase in the phosphorylation of DARPP-32 on Thr-34 (Svenningsson et al., 2000). This effect depended on a tonic activation of the cAMP pathway via D1 receptor or A2 receptor. In addition, in yeast, two-hybrid analysis has shown that spinophilin, but not the closely related protein neurabin, binds to the third intracellular loop of D2 receptors (Smith et al., 1999). This provides a possible link between D2 receptor and PP-1, the major target of Thr-34 phosphorylated DARPP-32, since spinophilin interacts with PP-1 (as mentioned earlier).

Activation of the D2 receptor can activate ERK in brain slices and in the striatal neurons in primary culture (Yan et al., 1999a; Brami-Cherrier et al., 2002). The D2-mediated ERK activation resulted from an elevation of intracellular calcium and the activation of PKC. In a variety of cells stably transfected with D2 receptors, D2 agonists were shown to activate ERK and JNK through Ras/MEK1- and SEK1-dependent mechanisms, respectively (Luo et al., 1998; Ghahremani et al., 1999). These activations involved Gi/o proteins since they were abolished by PTX treatment, and more precisely for ERK activation, $G\alpha_{i2}$ and $G\beta\gamma$ (Ghahremani et al., 2000). The activation of the Ras/MEK/ERK pathway can be the cause of cell growth and cell transformation produced by prolonged D2 receptor stimulation.

In contrast, in cell lines derived from the pituitary gland, especially those producing prolactin, D2 receptor activation prevents cell growth, reproducing the antiproliferative effects of D2 agonists on pituitary tumors of lactotroph origin (prolactinomas) (Saiardi et al., 1997; Arita et al., 1998). In D2 receptor-null mice, the incidence of prolactinomas was 100% in aged females, corroborating the negative control exerted by D2 receptors on the proliferative rate of lactotrophs (Kelly et al., 1997; Saiardi et al., 1997). The role of the ERK pathway is still unclear in this regulation. Although a similar antiproliferative effect was produced by the activation of D2 receptors in various cells derived from the pituitary gland, it seemed to result from an inhibition of ERK in GH4ZR7 cell line and primary rat pituitary cells, whereas it appeared to result from ERK activation in MMQ lactotroph cell line (Iaccarino et al., 2002; Liu et al., 2002). Moreover, the ERK pathway was down-regulated in D2 receptor-deficient mice and up-regulated in transgenic mice overexpressing D2S receptor in the pituitary gland (Iaccarino et al., 2002). Whatever the role of ERK, it is clear that the antiproliferative action of D2 receptors requires the D2S isoform of the receptor and the $G\alpha_o$ subunit, both of which are abundant in pituitary cells (Iaccarino et al., 2002; Liu et al., 2002). In GH4C1 cells transfected with D2 receptors, dopamine agonists have been reported to activate a tyrosine phosphatase activity, an action that may also contribute to the decreased cell division (Florio et al., 1992).

4.1.6. Action of D2 receptor on lipid metabolism

In many cell types, D2 receptor stimulation has an effect on enzymes metabolizing membrane lipids. We have mentioned above that in mesenchyme-derived cells and in striatal neurons, D2 agonists stimulate the activity of PLCβ by mobilizing $G\beta\gamma$ complex, and produce an inositol triphosphate-dependent Ca^{2+} release from intracellular stores (Ghahremani et al., 1999). In CHO cells stably transfected with D2 receptors, D2 agonists potently enhance the release of arachidonic acid when intracellular Ca^{2+} levels are already enhanced. This effect was observed following stimulation of various Gi/o-coupled

receptors transfected in CHO cells (Felder et al., 1991; Kanterman et al., 1991; Piomelli et al., 1991). The D2-mediated potentiation of arachidonic acid release was abolished by pretreating the cells with PTX and, interestingly, was enhanced by the activation of PKC and D1 receptors (Piomelli et al., 1991; Di Marzo et al., 1993). Recently, D2 agonists were found to activate phospholipase D, but not PLC, in GH4C1 cells transfected with D2 receptors (Senogles 2000). Surprisingly, the D2-mediated activation of phospholipase D was unaffected by treatment with PTX, but was abolished by the application of C3 exoenzyme, showing the involvement of small G proteins of the Rho family.

4.1.7. Do the short and long isoforms of D2 receptor regulate different signaling pathways?

The short (D2S) and the long (D2L) isoforms of D2 receptor are generated by alternative splicing from a single gene and differ by a 29 amino acid insert (Dal Toso et al., 1989; Giros et al., 1989; Grandy et al., 1989; Monsma et al., 1989). The D2L isoform is more abundant in the pituitary gland and all the brain regions with the exception of dopaminergic cell bodies and axons in which the ratio is inverted (Montmayeur et al., 1991; Khan et al., 1998). The two isoforms display only marginal differences in their pharmacological profiles (Malmberg et al., 1993), but the alternative insert is located within the third cytoplasmic loop that binds to G proteins and the two isoforms might interact preferentially with different G proteins and thereby initiate distinct intracellular signals. These distinctive features in signaling for D2S and D2L isoforms could explain why the behavioral effects of D2 agonists and antagonists are profoundly altered in mice in which D2L has been replaced by D2S using genetic recombination (Usiello et al., 2000; Wang et al., 2000). Interestingly, the D2-mediated inhibition of phosphorylation of DARPP-32 on Thr-34 residue was abolished in the striatum of these mutant mice suggesting that the D2S receptor was unable to replace D2L for triggering this effect in medium spiny neurons (Lindgren et al., 2003). In contrast, these mice displayed a normal inhibition of phosphorylation of tyrosine hydroxylase by D2 agonists, an event occurring in dopamine nerve endings that predominantly contain D2S receptors.

Although it has been suggested that D2S and D2L possess distinct G protein specificity for coupling to adenylyl cyclase, no agreement exists concerning the identity of the G proteins involved. One study reports that D2L interacts preferentially with $G\alpha_{i2}$ and another with $G\alpha_{i3}$ (Montmayeur et al., 1993; Senogles, 1994). In transgenic mice overexpressing either D2L or D2S isoforms in the pituitary gland, the ERK pathway appears to be differently regulated by the two isoforms (Iaccarino et al., 2002). However, the difference between D2S- and D2L-linked signaling seems relatively tenuous and the severe phenotype observed in the mutant mice with D2S substituted to D2L, is more likely to result from inappropriate targeting of D2S receptors in postsynaptic neurons, that compromises their ability to initiate intracellular signals (Usiello et al., 2000).

4.2. SIGNAL TRANSDUCTION OF D3 RECEPTORS

As compared to the D2 receptors, the signal transduction of D3 receptors was more difficult to study when these receptors were expressed by transfection in heterologous cellular systems. In general, it appears that D3 receptor stimulation results in the activation of most of the same effectors as in the case of D2 receptors. Nevertheless, the effects produced by D3 receptors are usually less pronounced than those produced by D2

receptors, probably explaining some negative results concerning the D3 receptor-mediated signaling pathways (Sokoloff et al., 1990; Freedman et al., 1994).

4.2.1. Coupling to G proteins

Numerous data suggest that D3 receptors possess a lower intrinsic ability to stimulate G proteins than D2 receptors. For most of the receptors with seven transmembrane segments, the binding studies reveal two molecular conformations for the receptor, one with a high affinity for agonists when the receptor is associated with G protein and another with a lower affinity when it is dissociated. The addition of GTP or analogues, by activating the G protein, promotes a dissociation of the G protein–receptor complex, appearing as a rightward displacement in the competition curves of antagonist binding with agonists. In CHO, COS7 or NG108-15 cells, nonhydrolyzable GTP analogues produce a much less pronounced rightward displacement with D3 receptors than with D2 receptors, an effect which may correspond to a lower capacity of D3 receptors to activate G proteins (Sokoloff et al., 1990; Chio et al., 1994a; Vanhauwe et al., 1999). The high-affinity conformation of D2 receptors usually exhibits a 100-fold higher affinity for dopamine than the low affinity conformation, whereas the ratio between the high and low affinities drops to 5–10 for the cloned D3 receptors in most studies (Levant, 1997). The D3 receptors appear to keep a relatively high affinity for agonists even in the presence of GTP analogues, this property being observed both in heterologous cell systems and in brain membranes (Levesque et al., 1992; Burris et al., 1994). D3 receptors are nevertheless coupled to G proteins, since their stimulation induced an increase in the binding of ^{35}S-GTPγS to membranes of cells stably transfected with D3 receptors (Robinson and Caron, 1997; Newman-Tancredi et al., 1999; Vanhauwe et al., 1999). However, whereas D2 receptor stimulation increased GTPγS binding by 300%, the stimulation of D3 receptor increased this binding by only 70% (Vanhauwe et al., 1999). The low efficiency of D3 receptor stimulation is not due to a preferential effect on a particular Gα_i subtype, since when D3 receptors were cotransfected with the various Gα_i subtypes in HEK293 cells, D3 receptor-induced effects on GTPγS binding were invariably low and similar for the various Gα_i subunits (Robinson and Caron, 1997).

4.2.2. D3 receptor inhibition of cAMP signaling

When the D3 receptor was transfected in CHO, NG 108-15 or *Xenopus* melanophore cells, dopamine agonists were found to inhibit the cAMP production (Chio et al., 1994a; Potenza et al., 1994; Griffon et al., 1997) by mechanisms involving PTX sensitive G proteins. In cells expressing similar amounts of D2 and D3 receptors, it appeared that the efficiency of D3 receptors to decrease cAMP was much lower than that of D2 receptors (Chio et al., 1994a; Vanhauwe et al., 1999). In HEK 293 cells, D3 receptor stimulation was unable to inhibit the forskolin-stimulated production of cAMP, in contrast with the pronounced effects of D2 receptor stimulation (Robinson and Caron, 1997). Interestingly, when the D3 receptor was cotransfected with the adenylyl cyclase isoform ACV, the dopamine agonist quinpirole potently inhibited cAMP production (Robinson and Caron, 1997). This effect was selective for this isoform of adenylyl cyclase, since it was not observed with ACI, ACII or ACVI (Robinson and Caron, 1997). This lack of effect was particularly surprising for ACVI since this isoform displays functional properties very close to ACV and this implied a high specificity for D3 receptors in inhibiting ACV.

These results could suggest a preferential association between D3 receptors and ACV and, interestingly, in limbic brain areas, ACV is highly expressed in the neurons that bear the D3 receptors (Diaz et al., 1995; Matsuoka et al., 1997).

4.2.3. Action of D3 receptors on ion channels

Like D2 receptors, the D3 receptors have the ability to regulate K^+ channels. When MES-23.5 cells, a mesencephalic cell line synthesizing dopamine was transfected with D3 receptors, dopamine agonists were found to increase K^+ currents (Liu et al., 1999). This effect was abolished by PTX and by anti-$G\alpha_o$ antibodies. However, $G\alpha_o$ may not be directly responsible for this effect, since in CHO cells or *Xenopus* oocytes, D3 receptor stimulation activated cotransfected GIRK channels via the recruitment of $G\beta\gamma$ complex, independently of the inhibition of cAMP production (Werner et al., 1996; Kuzhikandathil et al., 1998). D3 receptors also promoted the opening of GIRK channels in AtT-20 cells, which are derived from the pituitary gland cells and express endogenous GIRKs formed by Kir3.1 and Kir3.2 dimers. In these cells, the D3 receptor-stimulated K^+ current was abolished by a dominant negative mutant of Kir3.2 (Kuzhikandathil and Oxford 2000). When the efficiencies of D2 and D3 receptors were compared in *Xenopus* oocytes, the stimulation of D3 receptor induced three-fold lower effects than that of D2 receptor (Werner et al., 1996). Although the opening of K^+ channels usually induces a hyperpolarization of the cells and leads to an inactivation of voltage-dependent Ca^{2+} channels, it has been reported that D3 receptor stimulation activates high-threshold Ca^{2+} channels when transfected in NG-108-15 cells (Seabrook et al., 1994a).

4.2.4. Effects of D3 receptor on cell proliferation and Na^+/H^+ exchange

When transfected in CHO or NG 108-15 cells (Chio et al., 1994a; Pilon et al., 1994; Griffon et al., 1997), D3 receptor stimulation increased cell proliferation. This effect was abolished by PTX treatments showing the implication of Gi/Go protein. This effect was independent of inhibition of cAMP production since forskolin potentiated the D3 receptor-mediated proliferation (Schwartz et al., 1998). The intracellular events leading to the proliferative response are not well understood. D3 receptor stimulation was ineffective for stimulating the PLC activity, but the PKC stimulant PMA increased the proliferative response. This response could involve tyrosine kinase since genistein partly blocks the response (Griffon et al., 1997). In addition, activation of immediate early genes could be implicated since dopamine agonists stimulated c-Fos production in D3 receptor-transfected NG 108-15 (Pilon et al., 1994). In its third intracellular loop, the D3 receptor possesses a SH3 domain binding sequence that mediates D3 receptor interaction with Grb2 in vitro (Oldenhof et al., 2001). This interaction could potentially activate the Ras signal transduction pathway, leading to ERK activation but this mechanism remains to be proved in a cell model.

Like D2 receptors, when D3 receptors are transfected in heterologous systems, they are able to stimulate Na^+/H^+ exchange in the cells producing an acidification of the culture medium. This effect is due to the activation of the amiloride-sensitive Na^+/H^+ antiporter and is dependent on a Gi/Go protein activation and partly on the inhibition of cAMP production (Chio et al., 1994a; Vanhauwe et al., 1999). However, the D3 receptor stimulation appeared to be less efficiently than that of D2 receptor on this response (Chio et al., 1994a; Vanhauwe et al., 1999).

4.2.5. Conclusion

D3 receptors have coupling mechanisms qualitatively similar to D2 receptors, although this coupling seems to be less efficient. They may have a preferential ability to inhibit ACV with which they are coexpressed in the ventral striatum, and they have the possibility to interact directly with SH3 domain containing proteins, although the physiological role of this interaction remains to be established.

4.3. D4 RECEPTORS

Like the other members of D2-like receptor family, the D4 receptors are coupled to multiple intracellular effectors and almost all studies with heterologous expression systems conclude that functional coupling is dependent on the PTX-sensitive G proteins (Oak et al., 2000). In transfected cells agonist stimulation of D4 receptors was found to inhibit the adenylyl cyclase activity (Chio et al., 1994b; Asghari et al., 1995; Sanyal and Van Tol, 1997). The various polymorphic variants of the human D4 receptors, which differ by the number of sequence repeats in their third intracellular loop, display slight differences in their apparent affinity for dopamine but all have the ability to decrease cAMP production (Asghari et al., 1995). The D4 receptor-mediated inhibition of adenylyl cyclase activity was abolished by PTX treatment, although it has been shown that the D4 receptors can also couple the PTX-resistant $G\alpha_z$ and inhibit adenylyl cyclase activity in $G\alpha_z$-transfected COS-7 cells (Obadiah et al., 1999). In the in vitro experiments in which the D4 receptor was reconstituted with various $G\alpha_i$ subunits in cell membranes, the D4 receptor did not appear selective for any particular $G\alpha_i$ subunit (Kazmi et al., 2000). Somewhat surprisingly, in the mesencephalic cell line, MN9D transfected with mutated PTX-resistant $G\alpha_i$ or $G\alpha_o$ subunits, $G\alpha_{oA}$, $G\alpha_{oB}$ or $G\alpha_{1/2/3}$ failed to transduce the inhibition of cAMP production resulting from D4 receptor stimulation (O'Hara et al., 1996; Yamaguchi et al., 1997). However, the α subunit of transducin $G\alpha_{t2}$, which is sensitive to PTX, was shown to be expressed in these cells and the use of a toxin-resistant mutant of $G\alpha_{t2}$ in PTX-treated cells revealed that D4 receptors potently and preferentially activate $G\alpha_{t2}$ in MN9D cells (Yamaguchi et al., 1997). Interestingly, D4 receptor is present in the photoreceptor retina cells and the inhibition of cAMP production in dark-adapted rat retinas by D2-like agonists followed a D4 receptor pharmacology (Cohen et al., 1992). $G\alpha_{t2}$ appears to be expressed in other D4 receptor-containing tissues besides the retina and may well correspond to the preferential G protein for D4 receptor in the central and peripheral tissues (Yamaguchi et al., 1997).

As observed with the other D2-like receptors, the D4 receptors mediate additional signaling events that are independent of the changes in cAMP levels. Some of these intracellular signals can be transduced by the $G\beta\gamma$ complex. This is probably the case in HEK 293 transfected with a $G\beta\gamma$-sensitive adenylyl cyclase (ACII), in which activation of D4 receptor paradoxically produced a stimulation of cAMP production (Watts and Neve, 1997). In *Xenopus* oocytes, GIRK1 channels which can be stimulated by $G\beta\gamma$ subunits, were opened following the activation of D4 receptor (Werner et al., 1996; Pillai et al., 1998). In transfected GH4C1 cells, the D4 receptor stimulation was found to cause the blockade of voltage-sensitive Ca^{2+} channels (Seabrook et al., 1994b). This effect was attributed either to an increase in K^+ currents promoting a membrane hyperpolarization, or to a direct inhibition of Ca^{2+} channels by G proteins (Seabrook et al., 1994b). Similar to the D2 receptors, when D4 receptors are expressed in cells lacking L-type voltage-sensitive

Ca^{2+} channels, agonists produce transient increases in intracellular Ca^{2+} levels that probably result from the activation of the phosphoinositide hydrolysis and Ca^{2+} mobilization from intracellular stores (Kazmi et al., 2000).

In CHO cells, D4 receptors were shown to potentiate the increase in arachidonic acid release induced by ATP or Ca^{2+} ionophore. This effect appeared to require PTX-sensitive G proteins and to depend on PKC (Chio et al., 1994b; Huff 1996). In the same cells, D4 receptor stimulation produced H^+ excretion resulting from the activation of an amiloride-sensitive Na^+/H^+ exchanger through a PTX-sensitive mechanism (Chio et al., 1994b; Coldwell et al., 1999). D4 receptor stimulation also increased the proliferation of CHO cells (Huff, 1996), showing that the signal transduction pathways stimulated by D4 receptors are identical to those stimulated by D2 receptors in this cell type.

Like the D3 receptor, D4 receptor also contains putative SH3-binding sequences in its third intracellular loop and this loop was found to interact with several SH3 domain-containing proteins, including Grb2 and Nck (Oldenhof et al., 1998). The sequences interacting with SH3 domains were located in the regions flanking the polymorphic repeats in the human D4 receptors, but the polymorphic repeat itself was not essential for the interaction. In CHO cells, the activation of D4 receptors stimulated the phosphorylation of ERK (Oldenhof et al., 1998). The region of interaction with Grb2 was important for this effect since the mutant D4 receptors deleted for this region but which were still able to bind to agonists normally, did not promote activation of the ERK pathway (Oldenhof et al., 1998). However, this region of D4 receptors appeared to be essential for the inhibitory coupling to adenylyl cyclase also (Oldenhof et al., 1998).

In summary, the signaling pathways stimulated by D4 receptor appear to be very similar to those found for D2 and D3 receptors. The most striking difference concerns its ability to activate $G\alpha_{t2}$ protein which could confer on the D4-receptor signal transduction some particularities that remain to be clarified.

5. REGULATION OF GENE EXPRESSION BY DOPAMINE RECEPTOR SIGNALING

5.1. SIGNIFICANCE OF DOPAMINE-REGULATED GENE EXPRESSION

Both activation and blockade of dopamine receptors has been shown to cause changes in gene expression in striatal neurons (Robertson et al., 1989b; Dragunow et al., 1990; Graybiel et al., 1990). The intracellular signaling cascades produced by the stimulation (or inhibition) of dopamine receptors ultimately regulate the activity of transcription factors, resulting in the induction or repression of specific genes. Since these regulated genes can themselves encode transcription factors, the changes in dopamine transmission trigger a complex program of gene expression in the striatum (Berke et al., 1998). It has been proposed that the changes in gene expression cause certain dopamine-dependent alterations in neural functioning which have implications for human health. These alterations display a slow onset, but can have a prolonged duration. Addiction to psychostimulant drugs, which are indirect dopamine agonists, develops over a relatively long period of time after repetitive consumption. Antipsychotic drugs, all of which are antagonists of dopamine receptors, generally require several weeks of treatment before maximal therapeutic effects are achieved. It has been proposed that dopamine agonists and antagonists generate immediate effects which disappear with the drug,

as well as long-lasting changes within their target cells which are thought to require regulation of gene expression. Such long-lasting changes controlled by dopamine would include synaptic plasticity critical for both reward-controlled learning and development of addiction (Berke and Hyman, 2000; Hyman and Malenka, 2001).

The regulation of gene expression by dopamine receptors has been investigated by a variety of approaches in different models. Some studies analyzed the consequences of the lack of dopamine transmission occurring after neuroleptic treatment or after lesion of dopamine neurons, while others evaluated the effects of stimulation of dopamine neurotransmission by dopamine agonists or psychostimulant drugs in normal animals or in 6-hydroxydopamine (6-OHDA)-lesioned animals. Some results appear surprising since blockade and stimulation of dopamine receptors apparently produce the same effects on the expression of some genes. To understand these complex regulations, it is therefore necessary to identify their mechanisms at the level of the signaling pathways.

5.2. THE DOPAMINE-REGULATED GENES

The collection of genes the transcription of which is regulated by dopamine in the striatum, is probably relatively large. Using differential display PCR, more than 30 genes were found to be rapidly induced by a D1 receptor agonist treatment in the denervated striatum of animals with a unilateral lesion of dopamine neurons (Berke et al., 1998). The activation of the transcription of these genes was transient and for all the tested genes, the rate of activation of transcription returned to its normal levels 24 h after the treatment. The maximal activation occurred generally 1–2 h following the treatment but the induction of some genes, particularly those encoding dynorphin and tachykinin, presented a longer delay and a plateau of several hours. Interestingly the genes induced in these experimental conditions were also induced following treatment with either cocaine or the D2 selective antagonist eticlopride, suggesting that these various treatments activate the same pattern of genes (Berke et al., 1998).

Many activated genes in these conditions are immediate-early genes (IEGs), that are induced within minutes after treatment. IEGs often, but not exclusively, encode transcription factors including c-Fos, JunB, FosB, Fra-1, Fra-2, c-Jun, NAC1 and Egr1 (zif268, krox24, NGFI-A) (Robertson et al., 1989b; Graybiel et al., 1990; Hope et al., 1992; Moratalla et al., 1992; Cha et al., 1997). These transcription factors are presumed to play a key role in triggering a second wave of gene expression that underlies the long-lasting effects induced by changes in dopamine neurotransmission. Other IEGs encode proteins which affect more directly the function of the striatal neurons, including precursor proteins for neuropeptides (Gerfen, 2000a), signal transduction-related proteins (such as MKP1) (Berke et al., 1998), cytoskeleton-associated protein (such as Arc) (Lyford et al., 1995) and regulatory proteins of receptor (such as homer1a, a regulator of metabotropic glutamate receptors) (Brakeman et al., 1997).

5.3. ROLE OF THE cAMP PATHWAY AND CREB

The cAMP pathway is thought to play an important role in the gene expression regulated by the dopamine receptors. Indeed, many treatments that induce gene expression in the striatum are also known to increase cAMP levels. In the model of 6-OHDA-lesioned animal, gene induction was triggered by D1 agonist treatments or by L-DOPA via D1

receptor stimulation (Robertson et al., 1989a; Cole et al., 1993). The transcriptional effects of psychostimulant drugs, amphetamine and cocaine, were blocked by SCH23390, a selective blocker of D1 receptors (Graybiel et al., 1990). Neuroleptic administration could also activate the cAMP pathway by inhibiting the D2 receptors (Dragunow et al., 1990).

The CREB protein has been proposed to play an essential role in the cAMP pathway connecting dopamine receptor stimulation to the regulation of gene expression. CREB is a member of the family of leucine-zipper transcription factors (Shaywitz and Greenberg, 1999). Normally present in striatal cell nuclei, homodimers of CREB bind to specific DNA sequences, such as the cAMP-response elements (CRE), which are present in the promoter regions of many genes, including the dopamine-regulated genes encoding c-Fos, dynorphin and enkephalins. CREB is phosphorylated at Ser-133 by PKA and this promotes target gene expression via the recruitment of two coactivators CREB-binding protein (CBP) and p300 (Kwok et al., 1994; Lundblad et al., 1995). In striatal neurons in culture, CREB phosphorylation is stimulated by dopamine or forskolin (Konradi et al., 1994) and in vivo, CREB phosphorylation is enhanced by manipulations that presumably stimulate cAMP production in the striatum, including treatments with amphetamine or with the D2-prefering antagonist haloperidol in intact animals, as well as L-DOPA or D1 agonist administrations in dopamine-depleted animals (Cole et al., 1994; Konradi et al., 1994; Konradi et al., 1996). However, CREB can be phosphorylated at Ser-133 and activated by Ca^{2+}/calmodulin-dependent kinases, CaMKII and CaMKIV as well as by kinases of Rsk/MSK family that are activated by the Ras/ERK pathway (Deisseroth and Tsien, 2002). In dissociated striatal cultures, blockade of Ca^{2+} entry by NMDA receptor antagonists was found to reduce the ability of dopamine to induce phosphorylation of CREB (Konradi et al., 1996). In vivo, cocaine administration to unlesioned mice, and D1 agonist treatment in 6-OHDA-lesioned rats activated the ERK pathway (Valjent et al., 2000; Gerfen et al., 2002). This effect may well contribute to the phosphorylation of CREB via the activation of p90-Rsk and/or MSK kinases (Valjent et al., 2000; Gerfen et al., 2002). Interestingly, the role of CREB in the rewarding effects of cocaine has been investigated by overexpression of CREB or of a dominant negative mutant of CREB in the nucleus accumbens by microinjection of recombinant viruses (Carlezon et al., 1998). The overexpression of CREB inhibited the cocaine effects in a conditioned place-preference test, whereas overexpression of CREB mutant potentiated these effects. These experiments strongly suggested that CREB plays a role in the rewarding properties of cocaine.

5.4. AP-1 COMPLEX

Activating protein 1 (AP-1) complexes are formed by the heterodimerization of two leucine-zipper transcription factors belonging to the Fos and Jun families. They bind to a specific DNA consensus sequence called the AP-1 site, which is found in the promoters of numerous neuronally expressed genes and may activate or repress their transcription. c-Fos which was the most studied of these transcription factors, has a very low basal expression in the striatum but its transcription is dramatically stimulated in a variety of experimental conditions altering the dopamine transmission in vivo, including administration of amphetamine, cocaine (Graybiel et al., 1990; Hope et al., 1992; Nguyen et al., 1992), haloperidol, raclopride (Nguyen et al., 1992; Robertson and Fibiger, 1992) in intact animals, as well as administration of L-DOPA or D1 or D2 agonists in dopamine-denervated animals (Robertson et al., 1989a; LaHoste et al., 1993). CRE sequences are

present in the promoter region of *c-fos* gene (Sheng et al., 1990) and it has been proposed that the induction of *c-fos* in response to alterations in dopamine transmission results from the phosphorylation of CREB. However, there exists also some evidence that c-Fos can act upstream of CREB and it is unclear if *c-fos* can be activated independently of CREB (Sanyal et al., 2002). Following the administration of D1 agonist, the expression of other AP-1 complex components was also induced in dopamine-depleted striatum since, for example, FosB, fra2, c-Jun, junB, and junD genes were transcribed (Hope et al., 1994). The pattern of c-Fos expression varies depending on the drugs used. For instance, following amphetamine administration, c-Fos immunoreactivity was increased at higher levels in the striosomal compartment than in the matrix (Graybiel et al., 1990). Moreover, psychostimulant drugs induced *c-fos* gene transcription selectively in striatal neurons expressing high levels of D1 receptors, whereas neuroleptics activated *c-fos* in neurons preferentially expressing D2 receptors (Cenci et al., 1992; Robertson and Jian, 1995; Steiner and Gerfen, 1995). However, by yet unclear mechanisms, the concomitant stimulation of D1 and D2 receptors has synergistic effects on the expression of c-Fos, which probably occurs at the level of D1-rich neurons (Keefe and Gerfen 1995). Following repeated administration of psychostimulant drugs, the composition of AP-1 complexes changes because c-Fos is no longer induced and more stable isoforms accumulate in striatal cells (Hope et al., 1994). Delta-FosB appears to be the main component of AP-1 complexes in the nucleus accumbens following chronic cocaine treatment (Nestler et al., 1999). Since this transcription factor regulates the expression of proteins, such as GluR2 or CDK5, which have a crucial role in striatal neuron physiology, delta-FosB was hypothesized to mediate long-term effects of cocaine abuse (Nestler et al., 1999; Bibb et al., 2001). In transgenic mice, the overexpression of delta-FosB in the nucleus accumbens increased the acute and delayed responses to cocaine (locomotor activity and reinforcing property in a conditioned place preference test) probably by increasing the expression of GluR2-type AMPA receptors (Kelz et al., 1999). Surprisingly, similar alterations in the responses to cocaine were observed in the absence of Fos-B in knockout mice (Hiroi et al., 1997), showing that if FosB is clearly involved in the animal reactivity to cocaine, its mechanisms of action remain to be further clarified.

5.5. REGULATION OF NEUROPEPTIDES EXPRESSION

Dopamine agonists and antagonists as well as the disruption of dopamine neurotransmission by either lesion of dopamine neurons or reserpine treatment modulate the expression of neuropeptide genes in the striatum, including those encoding enkephalin, dynorphin, substance P, neurotensin, somatostatine and CART (Cocaine and Amphetamine Regulated Transcript) peptide (Tang et al., 1983; Young et al., 1986; Voorn et al., 1987; Bean et al., 1989; Weiss and Chesselet, 1989; Gerfen et al., 1990; Kuhar and Dall Vechia, 1999). However, these peptides are differentially regulated by dopamine. For instance, reduction of dopamine input by the lesion of dopamine neurons resulted in increased enkephalin expression, reduced substance P expression and no change in dynorphin expression (Gerfen et al., 1990). Conversely, stimulation of dopamine receptors by psychostimulant drugs increased both substance P and dynorphin (Steiner and Gerfen, 1993). The model that prevails for explaining this differential peptide regulation, is based on the segregation of the various peptides in specific subpopulations of striatal neurons and their regulation via either D1 or D2 receptors (Gerfen, 2000b). Enkephalin is expressed in a subgroup of medium-sized spiny striatal neurons preferentially containing

D2 receptors, whereas substance P and dynorphin are expressed in a neuron population preferentially containing D1 receptors.

The lack of D2 receptor stimulation causes the enhancement of enkephalin gene expression seen following chronic neuroleptic treatment or destruction of dopamine neurons. It has been proposed that AP-1 and CREB are responsible for this effect because haloperidol was shown to induce *c-fos* gene and promote the phosphorylation of CREB. Two CRE sites, including the one able to bind both CREB and AP-1 complex, are found in the promoter region of enkephalin gene (Comb et al., 1988; Hyman et al., 1989). In transfected striatal neurons in culture, stimulation of cAMP pathway stimulated the transcription of enkephalin gene and this effect appeared to require two CRE sites (Konradi et al., 1995). The reduction of D2 receptor stimulation induced the enkephalin gene clearly via the cAMP pathway and the phosphorylation of CREB, but the role of AP-1 transcription factor remains to be clarified (Konradi et al., 1995).

In various experimental models, dynorphin gene expression was increased in the striatum by mechanisms requiring the stimulation of D1 receptors (Gerfen et al., 1990; Steiner and Gerfen, 1993). Three CRE sites were found in the promoter region of dynorphin gene and were shown to be indispensable for a full induction of the dynorphin gene in response to D1 receptor agonists in striatal neurons in culture (Cole et al., 1995). An AP-1 site is also present in the dynorphin gene promoter and is used as a target for the gene activation by AP-1 complexes in neuroblastoma cells (Naranjo et al., 1991). It is thus probable that psychostimulant drugs produce an increase in dynorphin in the striatum via a cascade of events involving D1 receptor stimulation, increase in cAMP production, CREB phosphorylation and an increase in AP-1 complex.

6. CONCLUSIONS

For many years, the dogma has been that dopamine receptors, which are the seven-transmembrane domain receptors, exert their effects through heterotrimeric G proteins. Although this remains true, recent results suggest the direct association of some dopamine receptors with ionotropic receptors or with adaptor proteins. These interactions, the functional significance of which remains to be established may enrich the repertoire of signaling used by dopamine. Heterotrimeric G proteins activated by dopamine can act either directly on specific types of K^+ or Ca^{2+} channels, or, indirectly, on numerous targets through second messengers and complex intracellular pathways involving protein kinases and phosphatases. While cAMP plays a central and essential role in signaling by D1 receptors, the coupling of D2 receptors appears to preferentially involve the regulation of ion channels. In striatal neurons a number of specific proteins mediate the D1 signaling pathway, suggesting that it may be endowed with characteristic properties, selected by evolution. These properties are still poorly understood and may require a combination of experimental approaches and precise modeling studies to be integrated into our understanding of the physiology of striatal neurons. The activation of D1 or D2 receptors and, in some instances their coactivation, results in the simultaneous triggering of several transduction pathways that impinge on a number of cytoplasmic and nuclear targets. The current conception holds that cytoplasmic targets, including ion channels and receptors, account for most of the acute effects of dopamine that may be inhibitory and/or excitatory, depending on the type and basal state of the target cell, as well as on the other inputs that are activated at the same time. In contrast, nuclear targets, including

transcription factors, are thought to be essential for the long-lasting effects of dopamine, including its role in long-lasting synaptic plasticity. The combination of these short-term and long-term effects is likely to be important for the physiological role of dopamine in the control of motricity and in reward-controlled learning. The elucidation of dopamine-activated pathways may also be essential for understanding the delayed therapeutic effects of neuroleptics. Indeed, the precise function of dopamine, a typical 'slow-acting' neurotransmitter, has been difficult to understand at the cellular level. This has been helped by the molecular dissection of its action on signaling pathways. Much remains to be done, however, to correlate these findings at the molecular level with the role of dopamine transmission in physiology and pathology. One essential consequence of these efforts, is that they allow to identify novel therapeutic targets in signaling pathways that will have to be considered, besides the traditional approaches directed towards receptors, for treating neurological and psychiatric illnesses.

7. ABBREVIATIONS

ACI-X	I-X Isoforms of adenylyl cyclase
AKAP	A kinase attachment protein
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AP-1	Activating protein 1
ARPP-16/19/21	cAMP-regulated phosphoprotein, 16/19/21 kDa
CaMK	Ca^{2+}/calmodulin-dependent kinase
CART	Cocaine and amphetamine-regulated transcript
CDK5	Cyclin-dependent kinase 5
CK1/2	Casein kinase 1/2
CRE	cAMP responsive element
CREB	cAMP responsive element binding protein
D2S/D2L	Short/long isoform of D2 receptor
DARPP-32	Dopamine- and cAMP-regulated Phosphoprotein, 32 kDa
ERK	Extracellular signal-regulated kinase
GluR1/2	GluR1/2 subunit of AMPA receptor
Gα	α subunit of G protein
Gβγ	βγ complex of G protein
GIRK	G protein-regulated inward rectifier K^+ channels
G protein	GTP binding protein
IEGs	Immediate-early genes
JNK	cJun N-terminal kinase
L-DOPA	3,4-Dihydroxy-L-phenylalanine
LTP	Long-term potentiation
LTD	Long-term depression
MAPK	Mitogen-activated protein kinase
MEK	MAPK or ERK kinase
MSK	Mitogen- and stress-activated protein kinase
NMDA	*N*-methyl-D-Aspartate
NR1	NR1 subunit of NMDA receptor
NR2A/B	NR2A/B subunit of NMDA receptor
6-OHDA	6-hydroxydopamine

PDE	Phosphodiesterase
PKA	cAMP-dependent protein kinase
PKC	Protein kinase C
PKG	cGMP-dependent protein kinase
PLC	phospholipase C
PP-1	Protein Phosphatase 1
PP-2A	Protein Phosphatase 2A
PP-2B	Protein Phosphatase 2B or Calcineurin
PTX	Pertussis toxin
Rsk	Ribosomal S6 kinase
SEK	Stress-activated protein kinase

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CHAPTER III

The use of dopamine receptor knockout mice in understanding brain dopamine neurotransmission and sprouting in the nigrostriatal pathway

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ABSTRACT

The neurotransmitter dopamine is thought to play a major role in a number of physiological processes and pathological conditions including schizophrenia, Parkinson's disease, Huntington's disease and drug addiction. Dopamine elicits its effects by interacting with a number of receptors, each with specific binding profiles, signaling cascades and expression profile. However, because of the structural similarity of these receptors, specific ligands are not available. A number of genetically manipulated mice with targeted deletions of dopamine receptors have been generated in an effort to understand the role of defined dopamine receptors in the brain function. This review will initially concentrate on describing the major biological insights gained from the analysis of dopamine receptor knockout mice and will then focus on the use of dopamine receptor knockout mice as a tool to mechanistically dissect a rodent model system of sprouting in the nigrostriatal pathway.

KEY WORDS: Dopamine receptors; behavior; amphetamine; striatum; nucleus accumbens; neuropeptide; sprouting.

1. DOPAMINE AND DOPAMINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

The neurotransmitter dopamine is thought to play a pivotal role in the regulation of a number of physiological processes (Jaber et al., 1996). Moreover, abnormal dopaminergic neurotransmission has been implicated in a spectrum of brain diseases, such as schizophrenia (Seeman et al., 1984), Parkinson's disease (Hornykiewicz, 1966), Gille de la Tourette syndrome (Peterson, 1996) and Huntington's disease (Ginovart et al., 1997), and is thought to be involved in tardive movement disorders known to complicate chronic neuroleptic treatment (Youssef and Waddington, 1987; Kane et al., 1988). In addition, dopamine is important in mediating reward and enduring changes in dopaminergic neurotransmission may underpin addictive behaviors (Koob, 1992a).

Dopamine is a neurotransmitter in the neural pathways, originating in the hypothalamus and the midbrain (Lindvall and Bjorland, 1983). The nigrostriatal pathway projects from the midbrain substantia nigra pars compacta (SNpc) to innervate the dorsal striatum (caudate putamen; CPu), a structure associated with planning, initiation, and coordination of voluntary movement (Graybiel, 1990; Gerfen, 1992). Loss of dopaminergic neurons within this pathway explains most of the clinical features of Parkinson's disease (Hornykiewicz, 1966). The mesolimbic pathway arises in the midbrain ventral tegmental area (VTA) and innervates the ventral striatum (nucleus accumbens (NAcc) and olfactory tubercle), and parts of the limbic system (septum, amygdaloid complex and piriform cortex). This pathway is believed to be important for motivated behaviors including activity related to reward and in particular some of the positive, reinforcing properties of commonly abused drugs such as alcohol, cocaine, amphetamines and opiates (Koob and Bloom, 1988; Koob, 1992a,b; Di Chiara, 1995; White, 1996). The mesocortical dopaminergic pathway also originates in the VTA and projects to the frontal, cingulate and entorhinal cortices. These areas are involved in emotional, motivational and cognitive functions such as certain aspects of learning and memory (Le Moal and Simon, 1991; Civelli et al., 1993).

Like many neurotransmitters, dopamine binds to specific transmembrane receptors on the surface of target cells to elicit its effects. Five subtypes of dopamine receptors have been cloned (Bunzow et al., 1988; Dearry et al., 1990; Monsma et al., 1990; Sokoloff et al., 1990; Sunahara et al., 1990, 1991; Zhou et al., 1990; Van Tol et al., 1991). All dopamine receptors couple to intracellular heterotrimeric guanine nucleotide-binding regulatory proteins (G-proteins) that regulate the majority of cellular functions triggered by receptor activation. The G-proteins are composed of α-, β- and γ-subunits, which, in the basal state, are associated with guanosine diphosphate (GDP). However upon receptor activation, GDP is replaced by guanosine triphosphate, inducing a conformational change that releases the α-subunit and a βγ-subunit complex, both of which can interact with a variety of effectors. The α-subunits are highly heterogeneous, and to a large extent, determine the cellular effects of individual receptor activation. More than 20 α-subunits have been identified, and have been divided into four classes, based on structural and functional homology (Liu et al., 1994b). Several α-subunits from the classes of $G\alpha_s$, $G\alpha_{i/o}$ and $G\alpha_q$ are involved in dopamine receptor signaling. As most dopamine receptors can couple to more than one α-subtype, the binding of dopamine to a single receptor may activate multiple effectors, leading to a complex cascade of downstream signaling and cellular effects. In addition, the activated dopamine receptors can directly modulate ion fluxes, independent of G-protein coupling (Missale et al., 1998). The exact mechanisms involved in G-protein-independent effects are uncertain. Identifying the effects of individual dopamine receptors can be difficult, due to a heterogeneous mix of receptors in the majority of tissues and the lack of specific ligands. As a result, most research investigating the coupling properties of dopamine receptors relies upon the expression of receptors in a variety of cell lines. While this approach has the advantage of examining a pure population of dopamine receptors, its limitations, in particular by the inherent variance of cell lines, have produced many conflicting results.

The five dopamine receptors are subdivided into two classes, referred to as D_1-like (composed of D_1 and D_5 dopamine receptors; D_1R and D_5R, respectively), and D_2-like (D_2, D_3 and D_4 dopamine receptors; D_2R, D_3R and D_4R, respectively), based upon their homology and ability to activate or inhibit adenylate cyclase (AC), respectively (Sibley and Monsma, 1992; Jaber et al., 1996). Each dopamine receptor species is thought to mediate

its specific in vivo effects by virtue of its intrinsic pharmacological binding properties for dopamine, downstream second messenger systems and its expression profile, both temporal and spatial, within the nervous system. As with signaling pathways, the specific in vivo role of individual receptors has been difficult to establish because of the lack of receptor specific ligands, especially with selectivity within the D_1-like and D_2-like subfamilies respectively (Jaber et al., 1996). In particular, the available ligands do not distinguish between D_1R and D_5R, or between D_2R, D_3R and D_4R (Jaber et al., 1996). The recent development of dopamine receptor gene targeted mutant mice that lack specific receptor subtypes offers a powerful tool to evaluate the roles of these receptors in dopamine-mediated neural processes. There have been a number of previous reviews in this area (Drago et al., 1998a; Sibley, 1999; Waddington et al., 2001; Hiroi et al., 2002; Tan et al., 2003).

1.1. D_1 DOPAMINE RECEPTOR

Of the five mammalian dopamine receptors cloned, D_1R and D_2R are the most highly expressed in the adult striatum (Gerfen, 1992). Cells transfected with an expression construct encoding D_1R have a high affinity for the D_1R-preferring antagonist SCH-23390 and a low affinity for the D_2R antagonist spiperone (Jaber et al., 1996). One of the most robust signaling effects of D_1R activation is the stimulation of AC, causing the accumulation of adenosine 3′,5′-cyclic monophosphate (cAMP) in a variety of cell culture models (Dearry et al., 1990; Monsma et al., 1990; Zhou et al., 1990). The activation of AC by D_1R activation is generally assumed to occur through $G\alpha_s$ subunit, however $G\alpha_{olf}$, which is present in higher levels in the striatum than $G\alpha_s$ (Herve et al., 1993), may also contribute to the stimulation of AC in the CPu and NAcc (Corvol et al., 2001). Furthermore, certain γ-subunits in the $\beta\gamma$-complex may also play a role in D_1R-dependent regulation of AC. Activation of D_1R modulate calcium levels, through L-type channels, however the effectors involved in this process are unclear. Mobilization of intracellular calcium stores via the stimulation of phosphatidylinositol (PI) hydrolysis by phospholipase C (PLC), can be triggered by D_1R coupled to $G\alpha_q$ in some brain regions (Undie and Friedman, 1990; Jin et al., 2001), but is not seen in COS-7 or Chinese hamster ovary cells expressing D_1R (Dearry et al., 1990; Pedersen et al., 1994). Alternative cascades that may be involved in increasing intracellular calcium may include the activation of protein kinase C via a PLC-dependent mechanism, or through a protein kinase A-mediated activation of calcium channels (Rodrigues Pdos and Dowling, 1990; Liu et al., 1992b). Even less certain are the effects of D_1R activation on the arachidonic acid pathway and potassium channels with a number of conflicting results documented. D_1R can also effect Na^+/H^+ exchange by cAMP-dependent and -independent mechanisms but only in tissues of nonneural origin (Felder et al., 1990, 1993).

D_1R gene expression is detectable in the rat striatum from embryonic day 14 of development, with D_1R mRNA observed in the developing CPu, olfactory tubercle, and frontal, cingulate, parietal and insular cortices in addition to the developing epithalamus, thalamus, hypothalamus, pons, spinal cord and neural retina (Schambra et al., 1994). Although D_2R message was detected in the mesencephalic dopaminergic nuclear complex, D_1R mRNA was not present in the substantia nigra (SN), entopeduncular nucleus and globus pallidus (Guennoun and Bloch, 1992; Sibley and Monsma, 1992). The lack of mRNA in these sites, together with the presence of numerous binding sites for the D_1R

suggest that in these areas, the D_1R is present only on axonal processes associated with D_1R positive striatal projection neurons. The developmental significance of prenatal D_1R mRNA is debatable, given the lack of high levels of functional D_1R detectable by ligand autoradiography (Schambra et al., 1994; Jung and Bennett, 1996b) and the finding that the D_1R gene expression precedes dopaminergic innervation. The development of striatal dopaminergic function was further investigated by monitoring cocaine and apomorphine-mediated induction of the immediate early gene, zif 268, during striatal ontogeny. Cocaine induction of striatal zif 268 expression is known to be a D_1R-dependent process (Drago et al., 1996). The earliest induction of zif 268 was seen at embryonic day 20 suggesting that functional D_1Rs are established only late in the embryonic development (Jung and Bennett, 1996a).

In the adult brain, D_1R is expressed at a higher level than any other dopamine receptor (Jaber et al., 1996). In addition to its widespread expression in both the ventral and dorsal striatum, it is also detectable in the limbic system, hypothalamus and thalamus. In situ hybridization studies suggested that D_1Rs are preferentially expressed on substance P and dynorphin positive striatal neurons which project directly to the substantia nigra pars reticulata/entopeduncular complex (SN/EP) (the direct pathway), whereas enkephalin positive D_2R neurons project to SN/EP via the external segment of the globus pallidus and subthalamic nucleus (the indirect pathway) (Gerfen et al., 1990). The validity of this dual pathway model has been challenged by studies reporting a substantial degree of D_1R and D_2R colocalization on striatal projection neurons (Surmeier et al., 1992, 1993; Surmeier and Kitai, 1994). A number of studies have demonstrated that dopamine differentially regulates the expression of neuropeptides in the striatum by acting at D_1Rs or D_2Rs (Sibley et al., 1992). For example, depletion of striatal dopamine with 6-hydroxydopamine (6-OHDA) induced lesions of the nigrostriatal dopamine pathway in animal models of Parkinson's disease which results in a reduced expression of substance P and dynorphin and an increased enkephalin expression. Moreover, these changes may be selectively reversed with subtype specific agonist treatments, so that D_1R agonist treatment normalizes substance P and dynorphin levels whereas D_2R agonist treatment normalizes enkephalin expression (Gerfen et al., 1990). The predominant differential expression of D_1R and D_2R was confirmed using a complex double transgenic paradigm (Drago et al., 1998b). Mice were generated in which an attenuated form of the diphtheria toxin gene (*tox-176*) was expressed exclusively in D_1R positive cells. Transgenic mice expressing *Cre*, a site-specific DNA recombinase, were crossed with a second line in which a transcriptionally silenced *tox-176* gene was inserted into the D_1R gene locus by homologous recombination. D_1Rs were not detectable in mutants by in situ hybridization or ligand autoradiography whereas D_2R mRNA and protein was present in the striatum. In addition, substance P and dynorphin, neuropeptides normally expressed in D_1R positive striatonigral projection neurons were not detectable whereas, enkephalin, a marker found in D_2R positive striatopallidal projection neurons was expressed in the mutant brain.

1.2. D_1 DOPAMINE RECEPTOR KNOCKOUT MICE ($D_1R(-/-)$)

Gene targeting by homologous recombination was used by two groups (Drago et al., 1994; Xu et al., 1994a) to produce mutant mice which lack functional D_1Rs in an effort to create models to investigate the in vivo role of these receptors on developmental, anatomical, behavioral, neuropharmacological and electrophysiological aspects of dopaminergic

transmission. Drago et al. (1994) generated a nonsense mutation of the D_1R by the combined strategy of insertion of the neomycin phosphotransferase gene into a site located in the region of the D_1R gene that encodes the fifth predicted transmembrane domain and removal of 0.75 Kb of downstream coding sequence. The absence of functional D_1R was confirmed using in situ hybridization, ligand autoradiography, quantitative saturation binding analysis on striatal derived membrane preparations (Drago et al., 1994), immunohistochemistry for the D_1R protein, in situ transcription analysis and by electrophysiology in striatal tissue slices (Levine et al., 1996). The mutation caused growth retardation and increased mortality in $D_1R(-/-)$ homozygous mice. The cause of death in $D_1R(-/-)$ mice was associated with a generalized failure to thrive after weaning, with selective impairment of motivated behavior, such as drinking and eating, which could be partially avoided by late weaning, caging mutant animals together to remove competitive selection pressures from wild type (wt) mice and making flavor-enriched homogenized food readily available on the cage floor. This behavior is wholly consistent with known effects of dopamine, which plays a critical role in motivation and reward mechanisms. Growth retardation could not be explained by hypocalcemia, renal failure or growth hormone deficiency. $D_1R(-/-)$ mice caged in modified conditions were examined neurologically and found to have normal righting, placing and grasp reflexes. An examination of the locomotor activity in an open field showed no difference compared to sex matched wt controls, however $D_1R(-/-)$ mutants displayed fewer rearing events. Rearing is considered part of a rodent's repertoire of spontaneous exploratory activities. Consistent with this observation of an altered rearing behavior in mutant mice, pharmacological studies have shown that the frequency of rearing events can be modulated by D_1R ligands with D_1R agonists increasing and D_1R antagonists decreasing the number of rearing events (Hoffman and Beninger, 1985; Breese et al., 1987; Dreher and Jackson, 1989; Chandler et al., 1990).

A number of other groups have independently quantified spontaneous behavior in this line of D_1R mutants (El-Ghundi et al., 1996; Clifford et al., 1997; Smith et al., 1998). Clifford et al. (1997) described an ethological evaluation (Waddington et al., 1995) of the spontaneous behavior of $D_1R(-/-)$ mice. This approach quantifies discreet components of mouse behavior under 'naturalistic' conditions of initial exploration and subsequent habituation to a novel environment. Relative to wt mice, $D_1R(-/-)$ mice demonstrated a reduction in sniffing, free rearing and sifting and chewing of cage bedding/fecal pellets (Clifford et al., 1997). Furthermore, in contrast to the original publication describing this mutant line, moderate increases in locomotion and grooming were also observed. In contrast, Smith et al. (1998) showed no significant difference in total locomotor activity between $D_1R(-/-)$ mice and wt controls in an open field paradigm. There was however an increased latency between the placement of $D_1R(-/-)$ mice in the center of the open field and initiation of locomotor activity compared to both heterozygous ($D_1R(+/-)$) mice and wt controls. Furthermore, although $D_1R(-/-)$ mice were able to learn a simple odor discrimination task, they showed impaired initiation and often failed to complete trials. $D_1R(-/-)$ mice also showed evidence of learning impairment when assessed in a water maze task used to assess learning and memory in mice. The latency to locating a hidden platform did not drop with time suggesting poor learning and the time spent in the target quadrant after removal of the platform was comparable to time spent in the opposite quadrant suggesting poor retention. Poor performance in these tasks was not due to swimming disability or difficulty in visual orientation and probably reflected disturbances in the function of D_1R-dependent cognitive processes. The study by El-Ghundi et al.

(1996) also examined spatial learning and memory in $D_1R(-/-)$ mice using a Morris water maze hidden platform paradigm and found that $D_1R(-/-)$ mice learned significantly slower than the controls. In accordance with the observations of Smith et al. (1998), $D_1R(-/-)$ mice spent less time in the target quadrant when retested after removal of the hidden platform confirming that D_1R are involved in memory retrieval.

The $D_1R(-/-)$ mutant mice generated by Xu et al. (1994a) also showed growth retardation and were routinely weaned late but increased mortality seen in the other $D_1R(-/-)$ line was not described. Moreover, the $D_1R(-/-)$ mice were reported as hyperactive in an activity cage paradigm. Locomotor activity, as assessed by photo beam interruption, was quantified during the light and dark phases of the light-dark cycle. Although spontaneous locomotor activity measured during the dark phase of the light-dark cycle (the normally hyperactive phase of the rodent circadian rhythm) demonstrated a significant hyperactivity in $D_1R(-/-)$ mice, testing during the light phase (as assessed by Drago et al. (1994)) failed to reach statistical significance. As expected, the $D_1R(-/-)$ mice did not respond to the motor stimulant effects of a D_1-like receptor agonist or to the motor-suppressive effects of a D_1-like receptor antagonist. Assessment over a shorter period of observation during the light phase of the light-dark cycle (recorded in the vehicle treated mice during the habituation phase of the D_1R agonist study) showed that mutant mice were significantly more active than their wt controls. This lack of internal consistency regarding light phase basal locomotor activity in $D_1R(-/-)$ mice as identified in this study suggests that a range of variables other than genotype may impact on the experimental outcome, such as day of the examination, sex of mice, novelty of the environment and the use of the same cohort of mice in previous experiments involving painful drug administration. Interestingly, $D_1R(-/-)$ mice were subsequently confirmed to show greater locomotor activity than their littermate controls while habituating to a novel testing environment during the light phase of the light/dark cycle (Xu et al., 1994b). This difference in the novelty seeking behavior appears to be a major difference between the two D_1R knockout lines. In addition to variability in behavioral assessment methods, more fundamental differences may underlie the variability in mutant phenotype in mice generated in the two laboratories. Although both groups back-crossed chimeras with C57BL/6 mice to generate heterozygous mice with a hybrid 129/C57BL/6 genetic background, there were differences in the ancestral origin of the embryonic stem (ES) cells used to generate the homologous recombinant ES clones, as well as differences in the configuration of the targeting vectors. Extensive genetic variation among 129 lines is well documented (Simpson et al., 1997) and adds to the degree of potential phenotype variability between knockout mice generated in different laboratories. The influence of the genetic background on the phenotype of genetically manipulated mice (Crawley, 1996; Gerlai, 1996; Lathe, 1996) and in particular the potential effect of strain-specific 'modifier' genes on penetrance of a given mutation has been highlighted (Lander and Schork, 1994). Differences in genetic background may also explain differences in phenotype observed with the same strain in different laboratories. A failure to maintain background genetic diversity due to restrictive breeding programs may significantly bias the phenotype.

From a developmental perspective it was shown that the functional deletion of D_1R does not interfere with the development of dopaminergic neurons, but it does decrease the overall brain size (Drago et al., 1994; Xu et al., 1994a). The volume of the striatum in adult mice was formally quantified in normal and homozygous $D_1R(-/-)$ mice using stereological methods (Drago et al., 1998b). The total volume of the striatum was

estimated and compared with the medial habenula nucleus, part of the epithalamus. The medial habenula was chosen for comparison because its borders are precisely defined and this nucleus does not contain D_1R (Dearry et al., 1990). The volume of the $D_1R(-/-)$ striatum was reduced by 22% compared to the wt control group (Drago et al., 1998b). In comparison, the volume of the medial habenula nucleus of $D_1R(-/-)$ and wt mice was not statistically different (Drago et al., 1998b). These results suggest that the D_1R may have a specific role in cell division, an idea supported by the findings of a recent publication (Ohtani et al., 2003). This study found that the D_1R activation was critical in progenitor cells contained within the lateral ganglionic eminence progressing from G_1 to S phase of the cell cycle. Finally, the finding of transcripts specific for cells normally expressing the targeted allele in the brain of $D_1R(-/-)$ mice and mRNA for substance P (Drago et al., 1994), a neuropeptide which colocalizes with D_1R, suggests that the developmental and postnatal expression of D_1R is not essential for the birth, survival or subsequent correct integration of D_1R positive striatal projection neurons in the adult brain.

Significant changes were seen in brain neuropeptide expression in $D_1R(-/-)$ mice. Expression of specific mRNAs that colocalize with D_1R neurons in the striatum are reduced in $D_1R(-/-)$ mice. Specifically, dynorphin and substance P (Drago et al., 1994; Xu et al., 1994a) are expressed at considerably lower levels than in wt mice. In contrast, enkephalin, a neuropeptide that is expressed in D_2R-positive striatopallidal projection neurons remains unchanged (Drago et al., 1994). The specificity of the changes in neuropeptide expression profile is consistent with 6-OHDA lesioning studies in rat, in which down-regulated substance P and dynorphin expression is reversed by D_1R agonist administration (Gerfen et al., 1991) whereas correction of enkephalin overexpression requires a D_2R agonist.

The role of this receptor subtype in the mechanism of action of cocaine was investigated by studies on $D_1R(-/-)$ mice. Cocaine acts by inhibiting dopamine reuptake by the dopamine transporter (DAT), and thereby increases the amount of synaptic dopamine available for interaction with pre- and postsynaptic dopamine receptors (Caine and Koob, 1993; Giros and Caron, 1993; Steiner and Gerfen, 1995). The relative role of D_1R and D_2R in this response, however, has long been debated. Animals lacking D_1R are insensitive to the locomotor activating effects of cocaine although at high doses they displayed increased sniffing and grooming as well as behavior suggestive of excessive serotonin receptor activation (Xu et al., 1994a; Drago et al., 1996). Psychomotor stimulants such as cocaine are also known to alter gene expression in striatal neurons. Because these genes are induced with selective D_1R agonists (Robertson et al., 1990, 1992) and blocked with selective D_1R antagonists (Young et al., 1991; Steiner and Gerfen, 1995), D_1R have a major role in their regulation. Cocaine failed to induce the immediate early genes c-fos and zif 268 in $D_1R(-/-)$ mice whereas it increases substance P expression in an abnormal pattern (Drago et al., 1996). Substance P is normally expressed in the striatum, NAcc, olfactory tubercle and islands of Calleja. In wt mice, cocaine treatment increased the expression of substance P, predominantly in the dorsal and lateral striatum. In $D_1R(-/-)$ mice, basal levels of substance P mRNA are reduced in all sites where this neuropeptide is normally expressed. Cocaine administration enhanced substance P expression in the lateral striatum in $D_1R(-/-)$ mice and littermate controls. In contrast, only in $D_1R(-/-)$ mice was substance P up-regulated in the central striatum , suggesting that although some of the effects of cocaine on gene regulation are mediated via D_1R-dependent mechanisms, additional nonD_1R mechanisms are also involved (at least in the mutant). The c-fos mRNA findings described in this study were confirmed at the protein

level in a subsequent study (Moratella et al., 1996). Furthermore, both cocaine and amphetamines failed to upregulate Jun B and dynorphin whereas haloperidol, a D_2R antagonist was able to specifically induce catalepsy and striatal Fos/Jun expression in $D_1R(-/-)$ mice. This study confirms that if the correct cellular signal is provided, c-fos up-regulation can occur in the brain of the mutant mice, albeit in a different subpopulation of striatal spiny projection neurons.

Cocaine and dopamine mediated neurophysiology was examined within the NAcc, a part of the brain believed to play a major role in the locomotor enhancing and rewarding effects of cocaine and other drugs of abuse (White, 1990; Koob, 1992b). Electrophysiological studies demonstrated that in dopamine-sensitive NAcc neurons of $D_1R(-/-)$ mice, there is a significant decrease in cocaine's inhibition of glutamate-generated action potentials. Furthermore, the inhibitory effects of dopamine, SKF 38393 (a D_1-like agonist) and quinpirole (a D_2-like agonist) were essentially abolished, even though D_2R binding and serotonin-mediated effects were unchanged (Xu et al., 1994a). The lack of D_1R-agonist effect was confirmed with single cell recordings of striatal neurons in slice preparations in vitro (Levine et al., 1996). The lack of a quinpirole effect in $D_1R(-/-)$ mice was consistent with the idea that D_1R activation is required for postsynaptic expression of D_2R agonist effects (Walters et al., 1987).

The place preference paradigm was used to investigate cocaine addiction. In this experiment, normal mice given repeated small doses of cocaine convert their place preference to coincide with the location paired with the drug. In contrast to the genotype specific effect of cocaine on locomotion, this drug was seen to result in a change in place preference (presumably reflecting a positive rewarding effect) to an equal degree in wt, $D_1R(+/-)$ and $D_1R(-/-)$ mice (Miner et al., 1995). However the effects of cocaine cannot be generalized to other less rewarding agents, presumably because of the potency of cocaine to addict. In particular, $D_1R(-/-)$ mice have attenuated alcohol-seeking behavior (George et al., 1996) and SCH 23390 (a D_1-like receptor antagonist) reduced alcohol intake in $D_1R(+/-)$ mice and wt controls but had no effect on $D_1R(-/-)$ mice. $D_1R(-/-)$ mice were particularly sensitive to the effects of sulpiride in lowering ethanol intake. Moratella et al. (1996) also described an enhanced sensitivity to D_2R antagonists, in an independently generated line of $D_1R(-/-)$, suggesting that compensatory alterations in baseline D_2R mechanisms may be important.

Crawford et al. (1997) used $D_1R(-/-)$ mice to investigate the molecular mechanisms underlying behavioral sensitization to amphetamines. In this paradigm, intermittent exposure to amphetamine produces a progressive and enduring increase in the locomotor response to a fixed dose (Crawford et al., 1997). This enhanced behavioral response associated with prolonged exposure to a drug, even after a period of abstinence, is thought to mirror processes involved with drug addiction in humans. Drug-induced modifications in dopaminergic neurotransmission involving the ventral striatum are thought to be important in the long-term expression of behavioral sensitization (Robinson et al., 1988; Kalivas and Duffy, 1993; Wolf et al., 1994) whereas the induction phase is dependent on D_1-like receptor stimulation in the VTA (Vezina, 1996). $D_1R(-/-)$ mice did not show a day-dependent increase in locomotor activity following repeated amphetamine treatment, normally seen during the induction phase, but did show an enhanced response after a three day abstinence, although this enhancement was not as pronounced as seen in wt controls. In addition, biochemical analysis confirmed that the down-regulated protein kinase A activity seen in control mice during the induction phase did not occur in $D_1R(-/-)$ mice, confirming that alterations in dopamine receptor-mediated downstream mechanisms

occur in concert with the establishment of behavioral sensitization (Roseboom et al., 1990; Kalivas et al., 1992; Steketee, 1994).

1.3. D_2 DOPAMINE RECEPTOR

Two isoforms of D_2R are produced by alternative splicing, generating a long D_2 receptor (D_2L) and a shorter form (D_2S) that differ by 29 amino acids in the third intracellular loop (Dal Toso et al., 1989). As the importance of the third intracellular loop for selective coupling to specific G-protein/effector systems has been shown by studies on other receptors (muscarinic and adrenergic receptors) (Kubo et al., 1988; Cotecchia et al., 1992), it is not surprising that differences in the G-protein coupling exists between the two isoforms of D_2R.

Using a variety of cell lines, D_2R has been shown to couple to numerous G-proteins including $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$ and $G\alpha_z$. Depending on the cell type and isoform expressed, D_2R activation can lead to the inhibition of AC and cAMP production, activation of potassium channels, inhibition of L-type calcium channels, stimulation of PLC activity and calcium mobilization, potentiation of Ca^{2+}-evoked arachidonic acid release, stimulation of Na^+/H^+ exchange, and regulation of PI hydrolysis (Di Marzo et al., 1993; Mercier et al., 2001).

The differential coupling of the D_2R isoforms to G-proteins and their effectors has been demonstrated by differences in the inhibition of AC in a wide range of tissues and cell lines. When expressed in a fibroblast cell line, the short isoform is far more effective in reducing cAMP accumulation than D_2L (Hayes et al., 1992). The D_2L requires the presence of $G\alpha_i$ subunits, in particular $G\alpha_{i2}$, to effectively inhibit AC (Montmayeur et al., 1993; Guiramand et al., 1995), whereas D_2S couples to $G\alpha_{i1}$ and/or $G\alpha_{i3}$ and/or $G\alpha_o$ to influence AC activity (Lledo et al., 1992; Liu et al., 1994b).

Both isoforms induce a PI-linked mobilization of intracellular calcium, when expressed in fibroblast cells, identical in response and pharmacology, and sensitive to pertussis toxin (PTX) treatment, indicating that a coupling of $G\alpha_i/G\alpha_o$ proteins were responsible (Vallar et al., 1990). Activation of protein kinase C blocked D_2S-mediated increase in calcium, whereas D_2L response was considerably more resistant (Liu et al., 1992a). However, activation of D_2R in a dopaminergic cell line did not affect PI hydrolysis (Tang et al., 1994) or rat striatal slices (Kelly et al., 1988; Rubinstein and Hitzemann, 1990), but actually inhibited hydrolysis in pituitary cells (Simmonds and Strange, 1985; Enjalbert et al., 1990).

Despite the activation of D_2R mobilizing intracellular stores of calcium in certain cell lines, D_2R also reduces inward calcium currents (Vallar et al., 1990; Williams et al., 1990; Lledo et al., 1992; Seabrook et al., 1994b). Depending on the cell type, this may occur via receptor-induced changes of potassium channels, leading to changes in membrane potential; or through the activation of G-proteins that directly inhibit calcium channels (Baertschi et al., 1992; Lledo et al., 1992; Liu et al., 1994b).

D_2R activation also increases outward potassium currents, leading to cell hyperpolarization in a number of preparations (Castelletti et al., 1989; Vallar et al., 1990; Einhorn et al., 1991; Lledo et al., 1992; Kitai and Surmeier, 1993). Although the effect on potassium channels has been established as G-protein-dependent, the α-subunit involved appears to differ with the tissue used, as in the pituitary $G\alpha_{i3}$ plays an essential role (Baertschi et al., 1992; Lledo et al., 1992), whereas $G\alpha_o$ is involved in preparations from the rat mesencephalon (Liu et al., 1994a).

D_2R causes a potentiation of calcium-evoked arachidonic acid release through G-protein-dependent mechanisms involving PKC in a range of cells (Felder et al., 1991; Kanterman et al., 1991; Piomelli et al., 1991; Nilsson et al., 1998).

Although the activation of phospholipase D (PLD) has been implicated in mitogenesis, oncogenesis and regulation of metabolism, the G-protein-coupled stimulation of PLD is not well understood. In GH4C1 cells (cloned from radiation induced rat pituitary tumor), activation of D_2S stimulated PLD activity, however it is uncertain if this effect is G-protein linked (Senogles, 2000).

In a range of tissues, D_2R activation appears to accelerate Na^+/H^+ exchange via a pathway that does not involve the inhibition of AC or PTX-sensitive G-proteins (Neve et al., 1992). However in a directly opposite finding, activation of D_2R increases extracellular acidification in PTX-manner by inhibiting Na^+/H^+ exchange (Ganz et al., 1990), indicating the variability that exists between cell lines.

Therefore, activation of D_2Rs may regulate multiple signaling pathways. Different receptor isoforms can activate the same pathway, but with different efficiency or via distinct regulatory mechanisms, or may evoke variable responses in different cell lines. This indicates that D_2L and D_2S may have distinct physiological roles in vivo. However, these studies have also demonstrated the difficulty in extrapolating findings from the signaling pathways in cell lines to those of the native receptor in neurons.

1.4. D_2 DOPAMINE RECEPTOR KNOCKOUT MICE ($D_2R(-/-)$)

The developmental profile of the D_2R, the first of the dopamine receptors to be cloned (Bunzow et al., 1988), together with the results of studies characterizing the ligand autoradiography, functional coupling based on agonist induced decreases in cAMP levels and modulation of immediate early gene expression have been described (Jung and Bennett, 1996a,b) and suggest an important early role of D_2Rs in brain function. The D_2R is expressed at high levels in a number of brain nuclei including the striatum, cortex, limbic system and hypothalamus and in the dopaminergic midbrain projection neurons of the adult brain (Bunzow et al., 1988). $D_2R(-/-)$ mice, described by Baik et al. (1995) provided a unique opportunity to examine the physiological involvement of D_2R in dopaminergic transmission. Mice lacking functional D_2Rs were generated using a construct containing a genomic fragment in which exon two was deleted and replaced with a neomycin phosphotransferase selection cassette (Baik et al., 1995). In $D_2R(-/-)$ mice there is a small reduction in body weight, locomotion is impaired, fertility is reduced but postnatal mortality is not increased. Motor behavior is blunted, with bradykinesia, postural and gait abnormalities and evidence of cataplexy, a motor phenotype that broadly resembles D_2R antagonist treatment (Jackson and Westlind-Danielsson, 1994). The descriptions of these knockouts were broadly consistent with the findings of an in vivo antisense experiment in which intraventricular infusion of an oligodeoxynucleotide with a sequence complementary to the rat D_2R mRNA reduced rat striatal D_2R and elicited catalepsy and reduced spontaneous locomotor activity (Zhang and Creese, 1993). Autoradiographic studies with the D_2-like receptor antagonist iodosulpride in $D_2R(-/-)$ mice confirmed the null mutation, with residual binding sites identified only in the islands of Calleja, presumably corresponding to D_3R. The absence of D_2R was accompanied by alterations of gene expression. Enkephalin mRNA was increased by 40% in the striatum, while brain tyrosine hydroxylase (TH) steady state mRNA levels were unchanged, suggesting that at least at this level of ascertainment, the dopamine synthetic pathway was unaffected by the absence

of D_2R. The increased enkephalin expression parallels the changes seen in 6-OHDA treated rats although the minor decrease in substance P expression (15%) also seen in these mice was unexpected. In contrast, dynorphin expression was unchanged in $D_2R(-/-)$ mice confirming that the two neuropeptides (substance P and dynorphin) are independently regulated. Although complete absence of D_2R did not affect the expression of the other members of the dopamine receptor family (D_1R, D_3R and D_4R), there were compensatory changes in the expression of glutamic acid decarboxylase (GAD, the enzyme that synthesizes the inhibitory neurotransmitter gamma-amino-butyric acid-GABA), which increased in the striatum by 20% and in the cortex by 40%. Following this original description, two other groups published on independently generated $D_2R(-/-)$ mice (Kelly et al., 1997; Jung et al., 1999). A detailed immunohistochemical study of the basal ganglia was undertaken on $D_2R(-/-)$ mice backcrossed five generations onto a C57BL/6 genetic background (Murer et al., 2000). As predicted by the indirect/direct pathway model of basal ganglia circuitry, enkephalin mRNA increased in the striatum and GAD expression decreased in the globus pallidus of $D_2R(-/-)$ mice. In addition, cytochrome oxidase 1 activity and expression (a marker of mitochondrial respiratory chain activity and therefore of neuronal activity (Porter et al., 1994), was increased in the subthalamic nucleus. Unexpected findings were a significant decrease in striatal substance P mRNA, a direct pathway marker, and no change in GAD expression in the basal ganglia output nuclei (entopeduncular nucleus and substantia nigra pars reticulata). In addition, GAD expression was unchanged in the striatum of $D_2R(-/-)$ mice. Differences between these results and those of Baik et al. (1995) may be explained by the differences in the genetic background of the $D_2R(-/-)$ mice.

Like D_1R, D_2R have been implicated as playing a major role in the behavioral responses to the drugs of abuse (Koob, 1992a). The mesolimbic dopaminergic projection originating in the VTA has been shown to be important in opiate addiction. Injection of morphine into the VTA has been shown to augment self-administration behavior (Broekkamp and Phillips, 1979) and produce a conditioned place preference (Phillips and Le Piane, 1980). D_2Rs within the NAcc are important in opiate withdrawal (Harris and Aston-Jones, 1994). Unlike $D_1R(-/-)$ mice, which fail to show locomotor activation in response to cocaine administration (Xu et al., 1994a; Miner et al., 1995; Drago et al., 1996), morphine administration increased locomotion in both $D_2R(-/-)$ mice and wt controls (Maldonado et al., 1997). In addition, administration of RB 101 (a mixed inhibitor of enkephalin degrading enzymes) also induced hyperactivity in both groups. Although the behavioral manifestation of opiate withdrawal precipitated by the opiate antagonist naloxone were present in $D_2R(-/-)$ mice, morphine rewarding properties (tested with a place preference paradigm) were completely absent, whereas wt mice spent significantly increased time in the drug-associated compartment during morphine administration (Maldonado et al., 1997). Furthermore, the lack of rewarding effects of opiates was specific as the behavior of $D_2R(-/-)$ mice and wt controls was the same when food was used as a rewarding stimulus. The results of a number of studies therefore challenge the dogma that motor activation and motivational responses to highly addictive drugs such as cocaine and opioids are interrelated. Preservation of motivational responses of $D_1R(-/-)$ mice to cocaine (Miner et al., 1995) occurs in the absence of locomotor activation (Xu et al., 1994a; Miner et al., 1995; Drago et al., 1996) and the study by Maldonado et al. (1997) demonstrated that the absence of place preference is not dependent on the locomotor impairment of $D_2R(-/-)$ mice, as these mice maintain a motor response to endogenous and exogenous opioids. The results of the study of opiate

place preference of Maldonado et al. (1997) were complemented by experiments undertaken on an independently generated line of $D_2R(-/-)$ mice (Dockstader et al., 2001). This group examined the behavior of $D_2R(-/-)$ mice that had been backcrossed for five generations onto a C57BL/6 genetic background (as compared to the C57BL/6 and 129 hybrid genetic background of mice in the Maldonado study) and found that D_2R function is critical in mediating place preference, a surrogate marker of addiction, in opiate-dependent and withdrawn states but not in an opiate-naïve paradigm. The influence of genetic background in the morphine place preference paradigm was clearly demonstrated in this study by the observation that increasing the 129 strain contribution abolished morphine place preference in C57BL/6 wt mice. An intravenous lever pressing self-administration paradigm in the same line of $D_2R(-/-)$ mice confirmed that an intact D_2R was essential for morphine to act as a behavioral reinforcer (Elmer et al., 2002). Unlike control mice, $D_2R(-/-)$ mice trained to lever press for water reward showed no difference in lever pressing rates for different concentrations of morphine in a fixed ratio paradigm and did not increase their rate of lever pressing in a progressive ratio paradigm.

On the same theme of reward, a number of studies have used $D_2R(-/-)$ mice to examine the role played by the D_2R in modulating the reinforcing effects of ethanol (Phillips et al., 1998; Cunningham et al., 2000; Risinger et al., 2000). $D_2R(-/-)$ mice showed a reduced ethanol preference and intake in a two-bottle (ethanol vs. water) choice paradigm (Phillips et al., 1998). A place-conditioning task was subsequently used to establish that this was because of a reduction in the rewarding effects of ethanol in $D_2R(-/-)$ mice, as the reduced preference as measured by a choice paradigm could also have been due to enhanced rewarding effects from smaller quantities of drug. $D_2R(-/-)$ mice showed no evidence of ethanol induced place conditioning under conditions that reliably produced place preference in wt control mice, confirming that D_2R functionality is necessary for ethanol-mediated reward processes (Cunningham et al., 2000). Finally, an operant ethanol self-administration paradigm was used to assess ethanol intake at various concentrations with and without saccharin (Risinger et al., 2000). The same lever-pressing paradigm was used to assess food self-administration. $D_2R(-/-)$ mice responded less to all three reinforces suggesting that the D_2R pathway may have a fundamental role in motivated behavior.

A number of studies examined the issue of the identity of the pre-and postsynaptic dopamine receptor. As described earlier, the alternative splicing of the D_2R gene is responsible for generating two isoforms of this receptor (D_2S and D_2L), that have similar pharmacological profiles, but differ in their coupling to G-proteins, indicating that distinct roles may exist for the two D_2R isoforms. The next major advance in understanding the in vivo functions of the two D_2R isoforms came with the generation of a genetically engineered mouse $D_2L(-/-)$ in which the D_2S is present but the D_2L is absent (Usiello et al., 2000). Examination of $D_2L(-/-)$ mice showed that levels of D_2S transcripts were increased both in the striatum and SN. $D_2L(-/-)$ mice were more sensitive to the locomotor inhibiting effects of the D_2-like receptor agonist quinpirole. In vivo microdialysis experiments confirmed that quinpirole caused a relative reduction in baseline dopamine release in $D_2L(-/-)$ mice. These findings were consistent with the idea that quinpirole mediated inhibition of locomotion was caused by a reduction in dopamine release following interaction with presynaptic dopamine receptors. In contrast, haloperidol, a D_2-like receptor antagonist, failed to elicit cataplexy in $D_2L(-/-)$ mice. This was interpreted as being due to a lack of postsynaptic D_2L isoforms. As expected,

blockade of up-regulated presynaptic D_2R by haloperidol caused an increase in extracellular dopamine levels, but as there were no functional postsynaptic D_2L receptors, cataplexy, the net effect of the drug was not seen. The high level of D_2S transcripts detected in the striatum of $D_2L(-/-)$ mice using sensitive RNAse protection assays were unable to functionally compensate for the lack of postsynaptic D_2L isoforms. This finding confirms that the D_2R isoforms are functionally distinct entities with specific downstream signaling pathways. Finally, this study also showed that D_1R agonists had reduced effects in $D_2L(-/-)$ mice suggesting that the D_1R/D_2R cooperative interaction is predominantly a postsynaptic process (where D_1Rs are located) mediated through the D_2L isoform. A complementary study used intracellular electrophysiological recordings undertaken in the same line of mice to confirm the pivotal role played by the D_2S isoform in the regulation of the activity of midbrain dopaminergic neurons (Centonze et al., 2002). Dopamine and quinpirole caused membrane hyperpolarization and inhibited the firing of midbrain dopaminergic neurons in wt mice but not in $D_2L(-/-)$ mice or in mice lacking both D_2L and D_2S isoforms, confirming that D_2S receptors are functionally significant somatodendritic autoreceptors in midbrain dopaminergic neurons. The dual finding of upregulated expression of the D_2S isoform in the SN of $D_2L(-/-)$ mice and low level expression of D_2L in the SN of wt mice (Usiello et al., 2000) complicates the interpretation of this electrophysiological study.

A further study provided compelling biochemical evidence that the D_2S isoform is the predominant presynaptic player and the D_2L is the primary postsynaptic D_2R isoform. $D_2R(-/-)$ mice also provided a powerful tool to examine the role of specific D_2R isoforms in the regulation of protein phosphorylation of key proteins involved in dopaminergic neurotransmission. Lindgren et al. (2003) examined the regulation of the phosphorylation of presynaptic enzyme TH, the rate-limiting enzyme in dopamine synthesis, and the postsynaptic dopamine-and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) in striatal slices. Quinpirole-mediated reductions in TH phosphorylation and its enzyme activity were abolished in complete $D_2R(-/-)$ mice (i.e. mice lacking both D_2R isoforms) but maintained in mutant mice with intact presynaptic D_2Rs (i.e. $D_2L(-/-)$ mice that express D_2S) (Lindgren et al., 2003). The phosphorylation of DARPP-32 induced by D_1R agonists is inhibited by the D_2-like agonist quinpirole. Consistent with the postulated location of D_2S presynaptically and D_2L on the postsynaptic membrane, quinpirole had no effect on D_1R agonist mediated DARPP-32 phosphorylation in mice lacking D_2L isoforms (i.e. complete and $D_2L(-/-)$ mice) (Lindgren et al., 2003).

In addition to regulating TH phosphorylation and dopamine synthesis, the presynaptic D_2R has a major role in modulating pulse-mediated stimulation of dopamine release (Schmitz et al., 2002). Dopamine overflow, as detected by voltammetry, evoked by a single stimulus was reduced in amplitude and duration in $D_2R(-/-)$ mice compared to wt mice. This was due to an increase in dopamine uptake in $D_2R(-/-)$ mice. Quinpirole, a combined D_2R/D_3R agonist had no effect in $D_2R(-/-)$ mice confirming that it was the D_2R rather than the presynaptically expressed D_3R that had a primary role in regulating DAT activity. This interaction between the D_2R and DAT has been examined in *Xenopus* oocytes (Mayfield and Zahniser, 2001). Although investigators in this study coexpressed the long isoform of the D_2R (rather than the short isoform which is thought to be the relevant presynaptic D_2R isoform), together with DAT, it was shown that activation of D_2R enhanced dopamine transporter expression on the cell surface was G-protein dependent and voltage independent.

1.5. D_3 DOPAMINE RECEPTOR

The amino acid sequence of the D_3R, the second receptor within the D_2 subfamily to be cloned (Sokoloff et al., 1990), was similar to that of the D_2R (Sibley and Monsma, 1992) with an overall homology of 52% with the D_2R, increasing to 75% in the transmembrane domains. As with the D_2R (Fishburn et al., 1995) splicing variants have been reported for the mouse D_3R, but not for the human D_3R homologue (Giros et al., 1991; Fishburn et al., 1993). Despite the structural homology, there are significant differences in the signal transduction cascades linked to the D_2R and D_3R. In particular, D_3R activation only weakly inhibits AC, and then only in some cell lines (Chio et al., 1994a; Potenza et al., 1994; McAllister et al., 1995; Jaber et al., 1996). Furthermore, D_3Rs do not couple to PI hydrolysis in any cell line tested, are not involved in changes in potassium currents and do not cause a potentiation of calcium-evoked arachidonic acid release (Davila et al., 2003). Similar to D_2R, D_3Rs can reduce inward calcium currents (Williams et al., 1990; Seabrook et al., 1994a), and can effect Na^+/H^+ exchange in a PTX-dependent manner (Chio et al., 1994a). There has been limited research into which G-protein α-subunits are responsible for D_3 receptor effects, however it is likely to be subunits from the $G\alpha_i/G\alpha_o$ families.

The D_3R is expressed mainly in the mesocorticolimbic pathway with only low level expression in the striatum (Sokoloff et al., 1990; Bouthenet et al., 1991). Abundant mRNA is detected in the shell of the NAcc in neurotensin containing neurons, olfactory tubercle, islands of Calleja (Landwehrmeyer et al., 1993a,b), SNpc, VTA and the cerebellum (Diaz et al., 1995).

1.6. D_3 DOPAMINE RECEPTOR KNOCKOUT MICE ($D_3R(-/-)$)

The development of a mouse mutant with a targeted mutation of the D_3R gene was a major step forward in understanding the function of this dopamine receptor in vivo (Accili et al., 1996), particularly given the lack of drugs that specifically stimulate or block D_3R function. To generate mice lacking D_3R, a targeting vector was constructed in which the neomycin selection cassette was introduced into the second exon of the murine D_3R gene resulting in an interruption of the second intracytoplasmic loop and the failure of production of both D_3R splice variants. Lack of D_3R was documented using competitive iodosulpride ligand autoradiography. Preferential labeling of D_3R was achieved by attenuating the majority of D_2R labeling with the D_2R-selective ligand domperidone. Conversely, D_2R were visualized by quenching D_3Rs by coincubation of the iodosulpride with quinelorane, a D_3R-preferring ligand. D_3R-specific binding was absent in the islands of Calleja of $D_3R(-/-)$ mice, the only brain structure in which this receptor species is highly abundant (Landwehrmeyer et al., 1993a,b).

Neurologically, the $D_3R(-/-)$ mice displayed normal gait and coordination and were shown to have intact primitive reflexes (Accili et al., 1996). However, mice homozygous for the mutant allele were hyperactive in an open field test for exploratory behavior, with increased locomotor activity and rearing (Accili et al., 1996). Data derived from genetic ablation of the D_3R gene in mice were therefore consistent with pharmacological studies in which 7-OH-DPAT, a dopaminergic agonist, which binds preferentially to D_3R, inhibits locomotor activity (Svensson et al., 1994), whereas UH232, a D_3R-preferring antagonist, causes hyperactivity (Waters et al., 1993). Although the selectivity of these drugs remains controversial, these studies support the conclusion that hyperactivity in $D_3R(-/-)$ mice is the result of ablation of D_3R rather than the effect of compensatory changes.

The same mice were examined in two animal models for anxiety-like behavior: the open field test and elevated plus maze test (Steiner et al., 1997). When assessed in the open field test, $D_3R(-/-)$ mice entered the center significantly more than their wt littermates, suggesting an anxiolytic-like effect of the D_3R mutation. Increased number of center entries was not simply a reflection of increased locomotor activity in $D_3R(-/-)$ mice as these mice also showed a significant increase in the number of locomotor activity normalized center entries. Consistent with this finding, $D_3R(-/-)$ mice entered open arms of the plus maze significantly more often and for a longer period than their littermate controls. In support of these observations, early clinical studies suggested that D_2-like dopamine receptor blockers have anxiolytic properties (Standish-Barry et al., 1983). Furthermore, animal studies using the same experimental paradigm have demonstrated anxiolytic-like effects for D_2-class antagonists and anxiogenic-like effects with D_2-class agonists (Pich and Samanin, 1986; Costall et al., 1987; Puglisi-Allegra and Cabib, 1988; Rogers et al., 1994). In contrast, D_1-class agonists and antagonists had no effects on anxiety-related behavioral measures (Puglisi-Allegra and Cabib, 1988; Rogers et al., 1994).

Consistent with the documented expression of the D_3R in nigrostriatal projection neurons, $D_3R(-/-)$ mice were shown to have abnormal dopamine neurotransmission. The locomotor hyperactivity was associated with elevated extracellular dopamine levels as measured by in vivo microdialysis (Joseph et al., 2002). Evoked dopamine release studied in striatal brain slices showed that the effect of the D_2R/D_3R agonist quinpirole in inhibiting dopamine release was mildly reduced in $D_3R(-/-)$ mice confirming that this receptor at least participated in D_2-like dopamine autoreceptor functionality.

A number of studies have used mice with targeted deletions of more than one dopamine receptor gene in an effort to understand cooperative interactions between dopamine receptors. Jung et al. (1999) independently generated D_2R, D_3R and D_2R/D_3R double mutant knockout mice and found that the motor phenotype was more severe in $D_2R/D_3R(-/-)$ mice than in $D_2R(-/-)$ mice. Immunoprecipitation experiments confirmed that D_3R are upregulated in $D_2R(-/-)$ mice (Jung et al., 1999). These data suggests that the D_3R may partially compensate for the lack of D_2Rs. In contrast, the biochemical phenotype identified in relation to the striatal calcium binding protein calbindin-D (28k) was distinct in $D_2R(-/-)$ and $D_3R(-/-)$ mice. $D_2R(-/-)$ mice showed calbindin immunoreactivity confined to the cytoplasmic rim of striatal neurons, whereas $D_3R(-/-)$ mice showed reduced calbindin immunoreactivity in the ventral striatum, the part of the striatum where D_3Rs are normally expressed at high levels. The changes identified in $D_2R/D_3R(-/-)$ mice were simply the changes seen in $D_2R(-/-)$ mice added to the changes seen in $D_3R(-/-)$ mice (Jung et al., 2000).

Neurochemical changes were examined in $D_1R(-/-)$, $D_3R(-/-)$ and $D_1R/D_3R(-/-)$ double knockout mice in an effort to explore potential cooperative interactions between the D_1R and D_3R (Wong et al., 2003b). Dopamine D_1- and D_2-like receptors and GABA receptor levels were assessed by ligand autoradiography and D_1R, D_2R, enkephalin, dynorphin and substance P transcripts measured by in situ hybridization. In agreement with a number of previous studies (Drago et al., 1994; Xu et al., 1994a), $D_1R(-/-)$ mice had normal GABA levels, reduced dynorphin and substance P, and increased enkephalin mRNA and dopamine D_2-like binding. $D_1R/D_3R(-/-)$ mice showed a decrease in dynorphin and substance P but normal enkephalin expression, whereas dopamine D_2-like and GABA receptor binding were increased. Major changes therefore occur in substance P

and dynorphin expression in $D_1R(-/-)$ mice and these changes are unaffected by loss of D_3R. It was postulated that the upregulated dopamine D_2-like binding and enkephalin levels in $D_1R(-/-)$ mice may be due to decreased dopamine turnover as was demonstrated in a recent study (Parish et al., 2001). As the upregulated enkephalin expression was identified in $D_1R(-/-)$ mice (Drago et al., 1996; Wong et al., 2003b) but not seen in $D_1R/D_3R(-/-)$ mice, it was concluded that enkephalin upregulation is dependent on functional D_3Rs.

The behavioral phenotype of $D_1R/D_3R(-/-)$ mice was compared with wt, and with $D_1R(-/-)$ and $D_3R(-/-)$ mice using an ethologically based topographical technique (Wong et al., 2003a). Compared to wt controls, $D_1R(-/-)$ mice showed alterations in a number of behavioral topographies, including increases in sniffing and locomotion with reductions in rearing behavior and chewing. In contrast, $D_3R(-/-)$ showed increases in sniffing, locomotion, total rearing, rearing free and rearing to wall, with reductions in grooming. Thereafter, $D_3R(-/-)$ mice did not show the prominence of delayed habituation in several topographies of behavior that characterized $D_1R(-/-)$ mice. The topographical profile of $D_1R/D_3R(-/-)$ mice over both exploratory and habituation phases were essentially indistinguishable from that of their $D_1R(-/-)$ counterparts suggesting that there was no $D_1R{:}D_3R$ interaction in the regulation of exploratory behavior. Although a large number of neurochemical parameters were quantified (Wong et al., 2003b), the loss of D_1R and downregulated substance P and dynorphin expression is seen in both $D_1R(-/-)$ and $D_1R/D_3R(-/-)$ mice, raising the possibility that the phenotype may be due to composite changes identified in the expression of these three molecules.

A second group (Karasinska et al., 2000) has compared the phenotypes of the D_1R, D_3R and D_1R/D_3R double mutants. They found that line crossings and undifferentiated rearing events were reduced in $D_1R/D_3R(-/-)$ and to a lesser extent in $D_1R(-/-)$ mice, but were normal (line crossings) or increased (rearing events) in $D_3R(-/-)$ mice. In the elevated plus-maze, the only finding was a greater number of open arm entries in $D_3R(-/-)$ mice. There were a number of significant methodological differences between the two studies. Only male mice were used by Karasinska et al. (2000); furthermore, the behavioral approach adopted differed radically from the ethologically based, topographical approach used by Wong et al. (2003a). In addition, the source of the $D_3R(-/-)$ mice and breeding programs differed between the two groups. Collectively these differences in experimentation, each capable of influencing apparent phenotype (Crabbe et al., 1999; Waddington et al., 2001; Wahlsten et al., 2003) makes it difficult to legitimately compare the results.

1.7. D_4 DOPAMINE RECEPTOR

Of the three cloned D_2-like dopamine receptor subtypes, the D_4R has the highest affinity for the atypical neuroleptic clozapine (Van Tol et al., 1991). The D_4R is expressed at high levels in the medulla, amygdala, midbrain, frontal cortex and the striatum (Van Tol et al., 1991), brain regions implicated in reward, cognitive processes and psychosis. The D_4R signal transduction mechanisms appear to be similar to the D_2R. Activation of D_4R can inhibit cAMP accumulation in range of cell culture lines (Chio et al., 1994b; McHale et al., 1994; Tang et al., 1994; McAllister et al., 1995), but do not couple to PI hydrolysis. D_4R can affect ion fluxes within cells, through the reduction of inward calcium current and

the increase of outward potassium currents (Liu et al., 1994a; Seabrook et al., 1994b). D_4Rs are also likely to be involved in the potentiation of calcium-evoked arachidonic acid release caused by D_2-like receptors and can affect Na^+/H^+ exchange (Chio et al., 1994b).

1.8. D_4 DOPAMINE RECEPTOR KNOCKOUT MICE ($D_4R(-/-)$)

The generation of a mutant mouse containing a targeted disruption of the D_4R (Rubinstein et al., 1997) was a major milestone in understanding its role in complex behavior, as ligands with absolute D_4R selectivity were and remain unavailable. The targeted allele contained a deletion of the second exon and analysis of transcripts derived from the mutant allele confirmed that exons 1 and 3 were spliced together resulting in a shift in the reading frame and premature appearance of a termination codon. $D_4R(-/-)$ mice were fertile and of normal size and showed a sustained reduction in locomotion and rearing events in a novel and familiar environment. Although $D_4R(-/-)$ mice showed a modest reduction in horizontal and vertical motion as assessed in an activity monitor assay, paradoxically $D_4R(-/-)$ mice outperformed wt controls in a rotarod assay which is designed to test complex coordinated motor activity. Demonstration of a lack of functional D_4R protein in mutant mice was difficult owing to lack of D_4R-specific antisera or ligand. A complex four drug in vivo assay was used to show that the D_4R was the site of action of clozapine. $D_4R(-/-)$ mice depleted of endogenous dopamine by the simultaneous administration of a TH inhibitor and reserpine were shown to be more sensitive to the clozapine inhibiting effects of apomorphine-induced reversal of akinesia (Rubinstein et al., 1997).

An anatomical and pharmacological evaluation of $D_4R(-/-)$ mice failed to reveal any gross structural abnormalities or differences in binding of the D_1-like or D_2-like receptors, although there was evidence of compensatory dopaminergic overactivity in $D_4R(-/-)$ mice, as reflected in elevation of the level of the dopamine metabolite DOPAC under basal conditions. The elevation of DOPAC was shown to be due to increased conversion following enhanced synthesis of dopamine. An interesting observation was that $D_4R(-/-)$ mice displayed locomotor sensitivity to ethanol and the psychostimulant drugs cocaine and methamphetamine. Although, ethanol (Imperato and Di Chiara, 1986), cocaine and amphetamine (Camp et al., 1994) were known to cause an elevation of dopamine release in the NAcc, this study was the first to demonstrate that enhanced basal dopaminergic activity is associated with enhanced sensitivity to the locomotor stimulant effects of these drugs. The elevated dopaminergic activity may not however translate directly to enhanced reward. Indeed, elevation of synaptic dopamine levels by blocking dopamine breakdown with selegilene, a monoamine oxidase-B inhibitor, significantly reduced ethanol intake in normal mice but had no effect in $D_1R(-/-)$ mice (George et al., 1996) confirming that in addition to dopamine turnover, a range of dopamine receptors participate in ethanol reward processes.

A number of other assessments have been undertaken on the $D_4R(-/-)$ mice aiming to identify subtle behavioral differences. The behavioral responses of the $D_4R(-/-)$ mice to novelty were examined with approach avoidance paradigms (Dulawa et al., 1999) and $D_4R(-/-)$ mice were found to be significantly less behaviorally responsive to novelty, although responses in two of the assays could be interpreted as enhanced anxiety-like behavior (reduced center entries in an open field test and emergence from a cylinder in an open field). Interestingly, a subsequent study confirmed that anxiety-like behavior, such as reduced exploration of the open arms of the elevated plus maze and longer latencies to

explore illuminated compartments of the light/dark shuttle box is increased in $D_4R(-/-)$ mice (Falzone et al., 2002). As expected, the anxiolytic drugs midazolam and ethanol reduced this anxiety-like behavior.

Brain dopamine contributes to a form of sensorimotor gating known as prepulse inhibition (PPI) and disturbances of PPI occur in psychotic individuals and in some unaffected first order relatives of schizophrenics (Freedman et al., 1997; Braff et al., 2001). Amphetamine, which can produce psychosis also disrupts PPI (Ralph et al., 1999). Because of the clinical benefits seen in the treatment of psychosis with the atypical neuroleptic clozapine, a drug thought to be highly selective for D_4R, the relationship between PPI and this receptor subunit was assessed (Ralph et al., 1999). Phenotypic expression of PPI and the disruption of PPI produced by amphetamine were examined in D_2R, D_3R and D_4R knockout mice. Although no phenotypic differences were noted in PPI in drug naïve mice, amphetamine induced disruptions in PPI were seen only in $D_2R(-/-)$ mice (Ralph et al., 1999). $D_3R(-/-)$ and $D_4R(-/-)$ mice were shown to have responses similar to wt. This result was surprising given the clinical benefits of clozapine.

1.9. D_5 DOPAMINE RECEPTOR

The human D_5R gene was cloned (Sunahara et al., 1991) and found to encode a 477-amino-acid protein with significant homology to the cloned D_1R. As expected, the receptor had an affinity for drugs that bound to the D_1R but displayed a 10-fold higher affinity for the endogenous agonist, dopamine. D_5R stimulation activated AC activity and its low abundance transcripts were detected primarily within limbic regions of the brain (Sunahara et al., 1991). In addition to stimulating cAMP formation, many of the intracellular signaling pathways triggered by D_1R activation are also regulated by D_5R. These include modulation of intracellular calcium levels in a G-protein dependent manner (Lezcano and Bergson, 2002; Baufreton et al., 2003) and Na^+/H^+ exchange in tissues of nonneural origin (Felder et al., 1990, 1993).

Limbic expression of the D_5R was validated in a detailed binding study using the D_1-like receptor antagonist [^{3}H]-SCH23390 undertaken in $D_1R(-/-)$ mice (Montague et al., 2001). No binding was detected in the striatum, NAcc, olfactory tubercles or amygdala of $D_1R(-/-)$ mouse brains, whereas, low levels of D_1-like binding were identified in the hippocampus, a finding that was subsequently confirmed by binding studies done on hippocampal homogenates.

1.10. D_5 DOPAMINE RECEPTOR KNOCKOUT MICE ($D_5R(-/-)$)

The D_5R was the last of the dopamine receptors to be targeted (Hollon et al., 2002). The $D_5R(-/-)$ mice were not growth retarded and displayed a normal home cage behavior and locomotor activity and responses in a large number of behavioral assays including the rotarod test, acoustic startle response, PPI, Morris water maze, cued and contextual fear conditioning, elevated plus-maze and light dark anxiety paradigms. As expected, they had a reduced motor activating response to the D_1R/D_5R agonist SKF 81297 (Holmes et al., 2001). As D_1-like receptor antagonists are known to inhibit a number of cocaine mediated responses, the role of the D_5R in mediating the locomotor stimulating and discriminative stimulus effects of cocaine was assessed in a recent study (Elliot et al., 2003). As with $D_1R(-/-)$ mice (Drago et al., 1996), cocaine mediated locomotor activity was reduced in $D_5R(-/-)$ mice confirming that both D_1-like dopamine receptor subtypes participate in

this response to cocaine. In contrast, the discriminative stimulus effects of cocaine are likely to be mediated predominately through the D_1R because blockade of the effect with the D_1-like antagonist SCH 39166 produced similar effects in $D_5R(-/-)$ and wt counterparts (Elliot et al., 2003). Both $D_5R(-/-)$ (Hollon et al., 2002) and $D_1R(-/-)$ (Albrecht et al., 1996) mice are hypertensive. The hypertension seen in $D_5R(-/-)$ (Hollon et al., 2002) mice has been shown to be mediated by increased sympathetic outflow originating within brainstem centers.

2. SPROUTING OF DOPAMINERGIC AXONS

The behavioral and functional consequences of dopamine receptor activation have been extensively examined and reported and some of these studies have already been discussed above. However the regulation of the size of the terminal arbor of dopamine neurons is mediated by dopamine receptors. This phenomenon has recently been reported (Finkelstein, 2002) and has significant ramifications for the behavioral effects mediated by dopamine and also in the interpretation of studies examining dopamine neurotransmission. The following section is a discussion and review of the role of dopamine receptors on the regulation of sprouting of dopaminergic neurons and axons.

2.1. THE ROLE OF DOPAMINE RECEPTORS IN REGULATING SPROUTING OF DOPAMINERGIC AXONS

There is now substantial evidence that neurons in the adult central nervous system can form new synapses, neurites and branches (Raisman and Field, 1973; Fagan and Gage, 1990, 1994; Frotscher et al., 1997). Following injury to the striatum or SNpc, compensatory changes occur that suggest regenerative processes are at play (Agid et al., 1973; Hefti et al., 1980; Robinson et al., 1990; van Horne et al., 1992). These changes include the formation of new synaptic terminals, identification of growth-cone structures (indicating axonal sprouting), neurite formation, increased number of TH-immunoreactive (TH-IR) hypertrophic fibers penetrating the striatum, the upregulated expression of factors that support neurite outgrowth and cell survival, and normalization of dopamine levels (Zigmond et al., 1984; Onn et al., 1986; Hornykiewicz, 1993; Thomas et al., 1994; Blanchard et al., 1995, 1996; Wenning et al., 1996; Cheng et al., 1998; Ho and Blum, 1998; Batchelor et al., 1999; Liberatore et al., 1999; Finkelstein et al., 2000). Blanchard et al. (1996) showed that between 4 and 7 months after 6-OHDA lesioning, the density of TH-IR fibers in the CPu increased, suggesting sprouting of axons from spared nigrostriatal neurons. In addition, electron microscopic examination of the CPu revealed axonal sprouts, larger than normal axonal varicosities and immunoreactive growth cone-like structures. Subsequently this sprouting response of SNpc neurons to varying degrees of deinnervation was quantified (Finkelstein et al., 2000). The extent of sprouting of individual surviving axons correlated with the degree of cell loss in the SNpc (Finkelstein et al., 2000), such that the density of terminals in the striatum remained normal until the loss of SNpc neurons exceeded 80% (Finkelstein et al., 2000; Parish et al., 2001), reminiscent of Parkinson's disease, where symptoms do not become apparent until significant numbers of dopamine SNpc neurons are lost (Hornykiewicz, 1998). The other significant feature however was that although the remaining axons formed very large terminal arbors (up to 10 times normal size in some neurons), it appeared that the sprouting was regulated so as to maintain normal terminal density, because sprouting was

proportional to the size of the lesion. This implied that the extent of sprouting might be regulated to maintain normal steady-state dopamine levels in the striatum. If this was so, it was likely that dopamine receptors, either presynaptic on nigrostriatal terminals or postsynaptic on striatal neurons, would participate in mediating the extent of the sprouting response. The predominant receptor types expressed in the dorsal tier of the striatum (the target region for the SNpc) are the D_1R and D_2R (Bjorklund and Lindvall, 1984; Gerfen et al., 1987; Weiner et al., 1991; Missale et al., 1998), whereas only D_2R transcripts are identified in nigrostriatal neurons (Drago et al., 1998a). A stereological method for estimating arbor size, described by Parish and colleagues (Parish et al., 2001, 2002b) made it possible to explore this issue further. In this method, the total number of DAT-IR terminals in the dorsal CPu is estimated and divided by the number of TH-IR

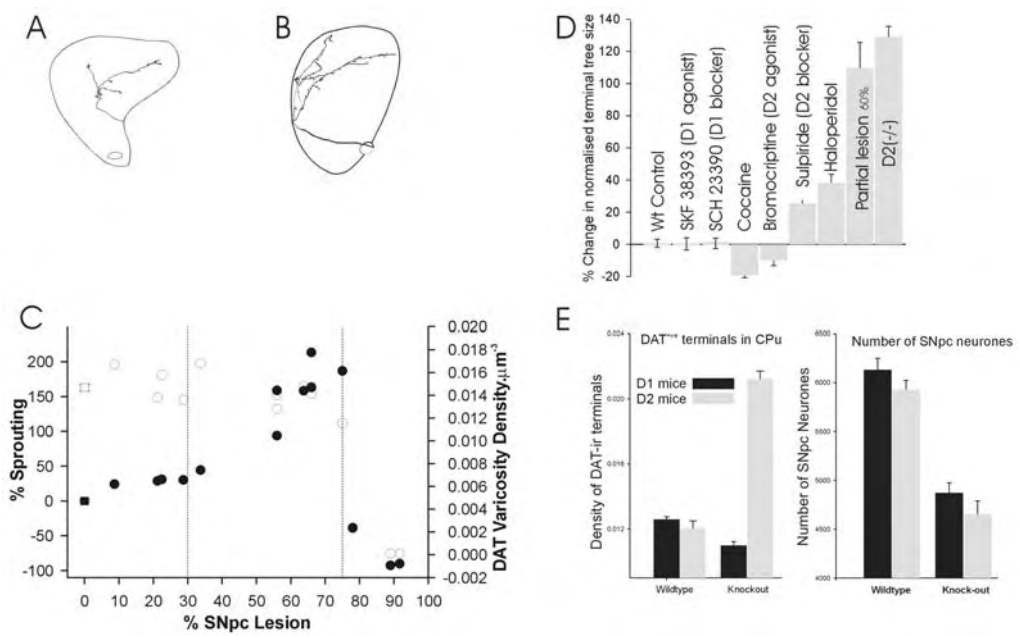

Fig. 1. To reconstruct dopaminergic axons in the striatum, the anterograde tracer dextran-biotin (DB) was injected into the SNpc. Single labeled SNpc axons were reconstructed and the morphology of each axon was quantified by counting the number of branching points and varicosities. Figure 1A is an axon from a normal animal and Fig. 1B demonstrates an axon from an animal who had received a partial lesion of the SNpc 4 months earlier. It demonstrates extensive sprouting in the animal with a lesion. In later studies, a stereological method was developed for rapidly assessing the extent of sprouting by estimating lesions size and terminal density. Figure 1C shows changes in density and sprouting of DAT-IR axonal varicosities in the dorsal striatum of rats with respect to lesion size. It shows the density of DAT-IR axonal varicosities (white symbols, left Y axis) and degree of terminal (varicosity) sprouting (black symbols, right Y axis) in the dorsal striatum 16 weeks after SNpc lesions. Square symbols represent data from control animals. The vertical lines indicate the point of division into small (0–30%), medium (30–75%) and large lesions (>75%). Various agents that selective activated dopamine receptors were administered over several months to examine their effect on tree size. This was compared with the tree size in various mutants (Figs. 1 and 1E). Drugs that selectively blocked D_2R caused an increase in tree size whereas drugs that activated the D_2R either directly or indirectly caused pruning of the tree. Mutants with selective deletion of the D_2R had the very large tree sizes. Deletion of the D_1R and drugs that selectively acted on the D_1R had no effect on tree size. Figure 1E provides a comparison of the density of terminals and number of dopamine neurons in the SNpc of wt and mice with selective deletion of the D_1R and D_2R. Figures from Finkelstein et al. (2000); Parish et al. (2001, 2002b).

neurons counted in the SNpc. Using this method, it was first confirmed that regeneration and sprouting following partial 6-OHDA lesions did result in the density of dopamine terminals in the striatum being maintained at normal levels (Fig. 1). Furthermore, in the intact animal, the terminal arbor expanded as a result of sprouting when agents that blocked the dopamine receptor were administered (Parish et al., 2001, 2002b). While the drugs used were relatively selective for the D_2R, suggesting a role for this receptor in regulating tree size, the availability of knockout mice, with targeted deletions of D_1R (Drago et al., 1994) and D_2R (Baik et al., 1995), provided powerful tools for unequivocally identifying the role of these two receptors in modulating the extent of sprouting in development and after partial loss of neurons in the SNpc of the adult brain (Parish et al., 2001).

The terminal arbor of drug naïve $D_2R(-/-)$ mice were 74% larger than normal, implying profuse axonal sprouting during development (Fig. 1). Furthermore, when exposed to long term receptor blockade or following lesions, $D_2R(-/-)$ mice were unable to mount a compensatory sprouting response, suggesting that they were unable to regulate their arbor size (Parish et al., 2001). In contrast, lesioning and long term administration of haloperidol and EEDQ resulted in an increase in terminal arbor size in $D_1R(-/-)$ mice (Parish et al., 2001). Furthermore, drug naïve $D_1R(-/-)$ mice had normal dopamine terminal density in the CPu, although because of reduced numbers of SNpc dopamine neurons, the calculated normalized terminal tree size suggested modest developmental sprouting. From these studies it was concluded that the D_2R was likely to be involved in regulating SNpc arbor size in development and following injury.

To further examine the role of the dopamine receptors in regulating SNpc arbor size, selective and nonselective D_1R and D_2R agonists and antagonists were administered to wt and to $D_1R(-/-)$ and $D_2R(-/-)$ mice. Pharmacological blockade of the D_2R resulted in sprouting of dopamine SNpc neurons in the wt mouse, whereas treatment with a D_2R agonist resulted in pruning of the terminal arbor of these neurons (Fig. 1). Agents such as cocaine, that indirectly stimulate D_2Rs, also resulted in a reduced terminal arbor in wt mice. Specific D_1R agonists and antagonists had no effect on the density of dopamine terminals in the striatum. Administration of dopamine agonists and antagonists had the same effects on $D_1R(-/-)$ mice as were observed in wt mice. In contrast, the terminal tree size of $D_2R(-/-)$ mice did not respond to either dopamine receptor agonists or antagonists or indirect acting dopamine receptor agonist cocaine, suggesting that they were unable to regulate their arbor size (Parish et al., 2001, 2002b).

It was thus concluded that D_2R has a major role in regulating the size of the terminal arbor in dopamine neurons projecting from the SNpc to the CPu. This is consistent with the role of the D_2 autoreceptor in regulating the delivery of dopamine. It suggests that this regulation is not only confined to dopamine storage, synthesis and turnover in the terminals but is also manifested in the density of dopamine terminals.

The D_2R exists in two forms; as an autoreceptor present on the presynaptic cell and as a postsynaptic receptor (Creese, 1982; Usiello et al., 2000). Because the $D_2R(-/-)$ mice used in this study lacked both slice variants, the identity of the D_2R isoform involved in the regulation of terminal arbor size and regenerative sprouting remains unknown. However, because the presynaptic receptor is expressed at a higher level and has a role in regulating the firing rate and propagation of action potentials as well as dopamine synthesis and release (see Section 2.2.5), it seems likely that this receptor is the major player regulating proliferation and sprouting in SNpc neurons. It is of interest that dopamine activity (calculated as a ratio of DOPAC and dopamine) was elevated in $D_2R(-/-)$ mice,

despite normal dopamine levels suggesting an impaired regulation of dopamine storage and release, as might be expected if D_2 autoreceptor functionality is impaired (see Section 2.5).

Although the focus of these studies was on the role of the neuronal dopamine receptors in regulating terminal tree size, other potential cytokine regulators of axonal growth were also investigated in addition to the role of specific nonneuronal cells especially as dopamine receptors are known to exist on glia (Khan et al., 2001) and oligodendrocytes (Bongarzone et al., 1998; Howard et al., 1998). Sprouting following both partial SNpc lesions and D_2R blockade is associated with microglial and astrocyte proliferation (Tripanichkul et al., 2001; Parish et al., 2002a,b) that extends well beyond the early inflammatory period associated with lesioning and also occur in animals treated with haloperidol only (where the reaction is not in response to injury). This late activation of glia is likely to support newly sprouted dopamine terminals by providing neurotrophic factors or scaffolding for growing neurites (Fallon et al., 1984; Noble et al., 1984; Fagan et al., 1997; Ho and Blum, 1997; Inoue et al., 1997; Goutan et al., 1998; Batchelor et al., 2000; Bresjanac and Antauer, 2000; McNaught and Jenner, 2000). Thus it is possible that sprouting is initiated or regulated by a D_2R-elicited glial response, which in turn leads to the release of growth factors, cytokines, scaffolding and a commensurate sprouting response.

2.2. IS POSTINJURY SPROUTING AND SPROUTING IN THE INTACT ANIMAL MEDIATED BY THE SAME MECHANISM?

Implicit in this discussion is the assumption that the D_2R antagonists-induced sprouting and sprouting in response to dopamine denervation in the striatum is orchestrated through similar mechanisms. In the case of the normal animal, where synaptic formations are intact, it has been argued that attenuated synaptic dopamine results in reduced presynaptic D_2R activation signals sprouting (Parish et al., 2002b). It is conceivable that in the case of reinnervation, growth cones, neurites or extending axons may have D_2R near their tips and lack of activation may be a stimulus to continue elongation (Koert et al., 2001). This question was addressed by combining these two treatments (lesioning and D_2R blockade) (Tripanichkul et al., 2003). Haloperidol administration caused a 57% increase in terminal tree size of dopamine nigral neurons projecting into the CPu. Following nigral lesions (causing a loss of less than 60% of dopamine SNpc neurons), terminal tree size increased by 51% on an average and returned the density of dopamine terminals to normal. However, administration of haloperidol for 16 weeks following lesioning resulted in reduced dopamine terminal density and terminal tree size (13%), consistent with absent or minimal sprouting. Thus, whereas D_2R blockade increases the density of immunoreactive SNpc terminals in the intact striatum, it prevents the increase induced by SNpc lesions. The switch between these two different D_2R effects appeared to be dependent on the establishment of synaptic contact. This conclusion was based on the coincident reappearance of synapses in the striatum and the switch in D_2R effect.

While the evidence for action through the D_2R seems solid in the first mechanism, it does open the possibility of an action through some other receptor type during injury. During development or repair, expression of D_3R and D_4R could increase as a compensatory response. In development, the D_3R subtype is expressed earlier than other

dopamine receptor subtypes. D_3R mRNA expression can be demonstrated in mice as early as day 9.5 postconception, whereas the D_2R subtype cannot be detected before day 13.5 postconception (Fishburn et al., 1996). It is therefore possible that during development (or injury) the D_3R (a dopamine receptor known to be expressed on dopaminergic neurons and therefore like the D_2R, a potentially presynaptic autoreceptor) may play a role in axon guidance. Central to the hypothesis regarding the role of D_2 autoreceptor in regulation of sprouting is the release and detection of dopamine by the presynaptic terminal. In this model, synaptic dopamine levels may be the important factor mediating arbor size, so that when there is reduced dopamine release, signaling through the D_2 autoreceptor not only results in upregulation of dopamine synthesis and release (Elsworth and Roth, 1997) but also increase in arbor size. During regeneration of axons through sprouting, normal synaptic structure is lacking and the highly regulated mechanism implied in this hypothesis seems unlikely. However, growth cones contain and release transmitters before establishing synaptic contacts (Taylor et al., 1990) and it has been suggested that neurotransmitters that are expressed throughout regeneration are directly involved in the regenerative response (Hokfelt et al., 1994; Zigmond et al., 1996; Zigmond and Sun, 1997; Shadiack et al., 2001; Zigmond, 2001). Indeed growth cones can be induced to turn toward the transmitter source (Zheng et al., 1994, 1996) or collapse and/or turn away from a transmitter source (Haydon et al., 1984; Lankford et al., 1987). Growth cones not only respond to exogenously applied transmitters but also to self released neurotransmitters such as serotonin and dopamine, which have been proposed to actively 'push' neurites to their targets (Hume et al., 1983; Young and Poo, 1983; Sun and Poo, 1987; Todd, 1992; Swarzenski et al., 1994; Spencer et al., 2000; Koert et al., 2001). In this case, neurite outgrowth would be specifically dependent on autoreceptors such as the D_2R (also see discussion on neurogenesis).

2.3. TIME COURSE OF SPROUTING

It seems likely that following partial SNpc lesions most (80%) dopamine terminals disappear from the dorsal striatum and subsequently, re-innervation proceeds by surviving SNpc neurons sprouting axons that travel along the medial forebrain bundle (MFB) to re-innervate the dorsal striatum (Fig. 2) (Stanic et al., 2003a). In medium-sized partial lesions, most (73%) of terminal labeling (DAT and TH) has disappeared from the dorsal striatum within four weeks of lesioning (Stanic et al., 2003a) with only occasional hypertrophic DAT-IR axons identified in the dorsal striatum, indicative of axons undergoing compensatory sprouting (Song and Haber, 2000). Because the proportion of terminal loss exceeded the proportion of neuronal loss, it is likely that terminals have degenerated from most arbors of SNpc neurons, not just from those of necrotic neurons. Furthermore, the reduction in terminal density was evenly distributed throughout the dorsal striatum rather than in clusters associated with surviving fibers. It is also unlikely that loss of TH and DAT immunoreactivity is due to downregulation or loss of DAT or TH expression rather than terminal degeneration. Loss of DAT-IR and TH-IR induced by intrastriatal 6-OHDA injections closely correlates with terminal degeneration demonstrated by silver staining (Hastings et al., 1996; Rabinovic et al., 2000), which labels degenerating cellular elements (DeOlmos and Ingram, 1971) and similar results were also observed in monkeys, five weeks after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment (Song and Haber, 2000).

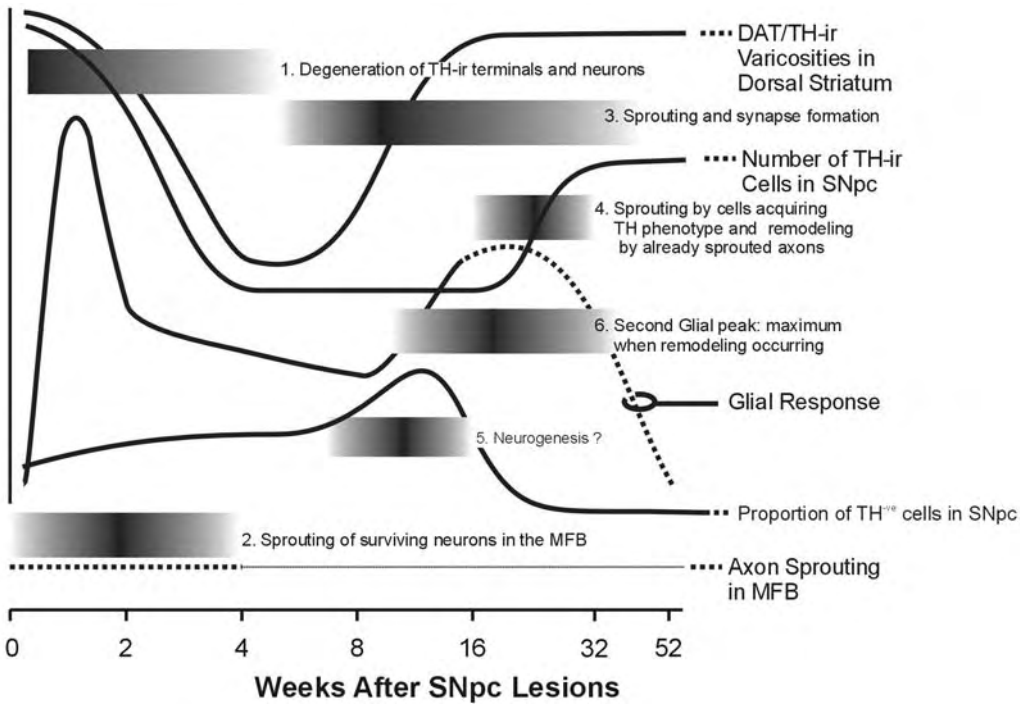

Fig. 2. Cartoon of events that occur following medium sized lesions of the SNpc. (1) The degenerative phase of varicosities and fibers in the dorsal striatum occurs in the 0–4 week period after SNpc lesioning and coincides with loss of neurons in the SNpc. (2) Axonal proliferation in the MFB was observed 2–4 weeks after lesioning. (3) DAT/TH-IR varicosities begin to increase 4 weeks after lesioning and density returns to normal by 16 weeks. We presume this represents sprouting of newly arrived axons in the dorsal striatum, which originated from surviving SNpc neurons. (4) At the same time as the reappearance of DAT/TH-IR varicosities there is an increase in the number of TH^{-ve} cells in the SNpc that is followed, some weeks later, by (5) an increase in TH-IR cells. The origin of the TH^{-ve} cells is unclear. If the cells that have newly acquired a TH phenotype 16–32 weeks after lesioning have sent axons into the striatum, then there is presumably a period when the arbors of pre-existing axons shrink and new synapse are formed by the new arrivals. (6) The glial response associated with sprouting is biphasic response with the first peak most likely associated with removal of degenerating debris while the late peak occurs when new synapses are being formed. Figure from Stanic et al. (2003a).

In the rat, terminal density begins to increase by about 4 weeks and but returns to normal density by 16 weeks after lesioning (Fig. 2) (Stanic et al., 2003a). It is likely that this reinnervation is from axon sprouting from the SNpc, tracking down the MFB and reinnervating the CPu. Stanic et al. (2003a) described a growing front of axons in the MFB between two and four weeks after lesioning (Fig. 2) and individually reconstructed axons had increased terminal arbors (Finkelstein et al., 2000). There are many descriptions of large numbers of TH and DAT-IR neurites entering the striatum following SNpc injury, indicating substantial reinnervation and presumably new synapse formation (Pickel et al., 1992; Thomas et al., 1994; Blanchard et al., 1995; Anglade et al., 1996; Blanchard et al., 1996; Ingham et al., 1996; Parish et al., 2001) and in the re-innervated dorsal striatum all dopamine terminals identified are morphologically different to terminal in normal rats (Finkelstein et al., 2000; Stanic et al., 2003b). It thus seems most likely that terminals reinnervating the striatum are newly formed from axons sprouting from existing or newly generated SNpc neurons.

2.4. WHAT CELLULAR ELEMENTS SPROUT?

Stanic et al. (2003a) identified sprouting axons in the MFB as early as two weeks after a lesion, yet Blanchard et al. (1996) observed growth cones entering the striatum seven months after partial lesions. Examination of the time course of reinnervation provided by Stanic et al. (2003a) implies that reinnervation may commence as early as two weeks, but that reestablishment of synapse is most prolific between 4 and 12 weeks, yet the recovery of the TH phenotype is on going and is perhaps most active at 16 weeks after lesioning. This suggests that not only does axon sprouting and re-innervation continue for many months, but also the source of innervation may alter over this time.

Recently it was shown that neurogenesis contributes to a steady low level of turnover of dopaminergic neurons in the normal rodent SNpc (Zhao et al., 2003). While the rate of neurogenesis was of several orders of magnitude less than in the granular cell layer of the dentate gyrus of the hippocampus, it was still sufficient to completely repopulate the nigra within the life time of the animal. Significantly, following an MPTP lesion, there was two fold increase in the rate of neurogenesis (Zhao et al., 2003). It is thus possible that neurogenesis contributes to the regenerative response through the sprouting of nascent neurons. Following 6-OHDA lesions there is an abrupt drop in the absolute number of SNpc neurons, reaching its lowest point at four weeks (Fig. 2). However, the proportion of SNpc neurons that are TH-IR falls even more than total number of SNpc neurons, reducing from the normal level of 90% to as low as 22% (Stanic et al., 2003a). The proportion of TH-IR neurons in the SNpc reached its nadir at about eight weeks after lesioning and recovers to normal proportions about 16 weeks after the reappearance of DAT-IR terminals in the dorsal striatum. During that time, both the proportion and the absolute number of TH^{-ve} neurons in the SNpc increases (Fig. 2). These TH^{-ve} cells may be the product of neurogenesis poised to express the dopamine phenotype once synaptic contact has occurred. Some recent reports would argue against this (Kay and Blum, 2000; Lie et al., 2002), although in those studies animals were killed six weeks after SNpc lesions, which may be too early to detect the establishment of nascent cells (Zhao et al., 2003).

Although repair can occur after small and medium lesions, it is puzzling as to why reinnervation after major lesions is at best modest. It seems unlikely that it is due to the extent of axonal retraction, as this appears to be extensive even after medium-sized lesions. As discussed earlier, remaining axons may be required to provide neurotransmitters or other guidance molecules for axons to track along (Parish et al., 2001; Tripanichkul et al., 2003) and following extensive injury there may be too few surviving axons to provide this guidance. This question requires further attention because its implications for repair after injury is obvious, whether it be from endogenous cells or by stem cells.

As discussed above, haloperidol may act to inhibit axon growth when administered soon after a lesion (Tripanichkul et al., 2003). However, it is also possible that it prevents the acquisition of the TH phenotype of nascent neurons. Whether or not the TH^{-ve} neurons in the nigra are the product of neurogenesis or loss of TH phenotype, they are present in increased numbers and they clearly undergo a dopaminergic phenotype acquisition with time. By acting through the D_2 family of receptors, dopamine can have varying effects on the differentiation of dopamine neurons. Dopamine can inhibit differentiation of retinal neurons (Guimaraes et al., 2001), promote survival of olfactory dopamine neurons but seemingly, have no effect on development or survival of mesencephalic neurones (Van Muiswinkel et al., 1993; Feron et al., 1999). As discussed

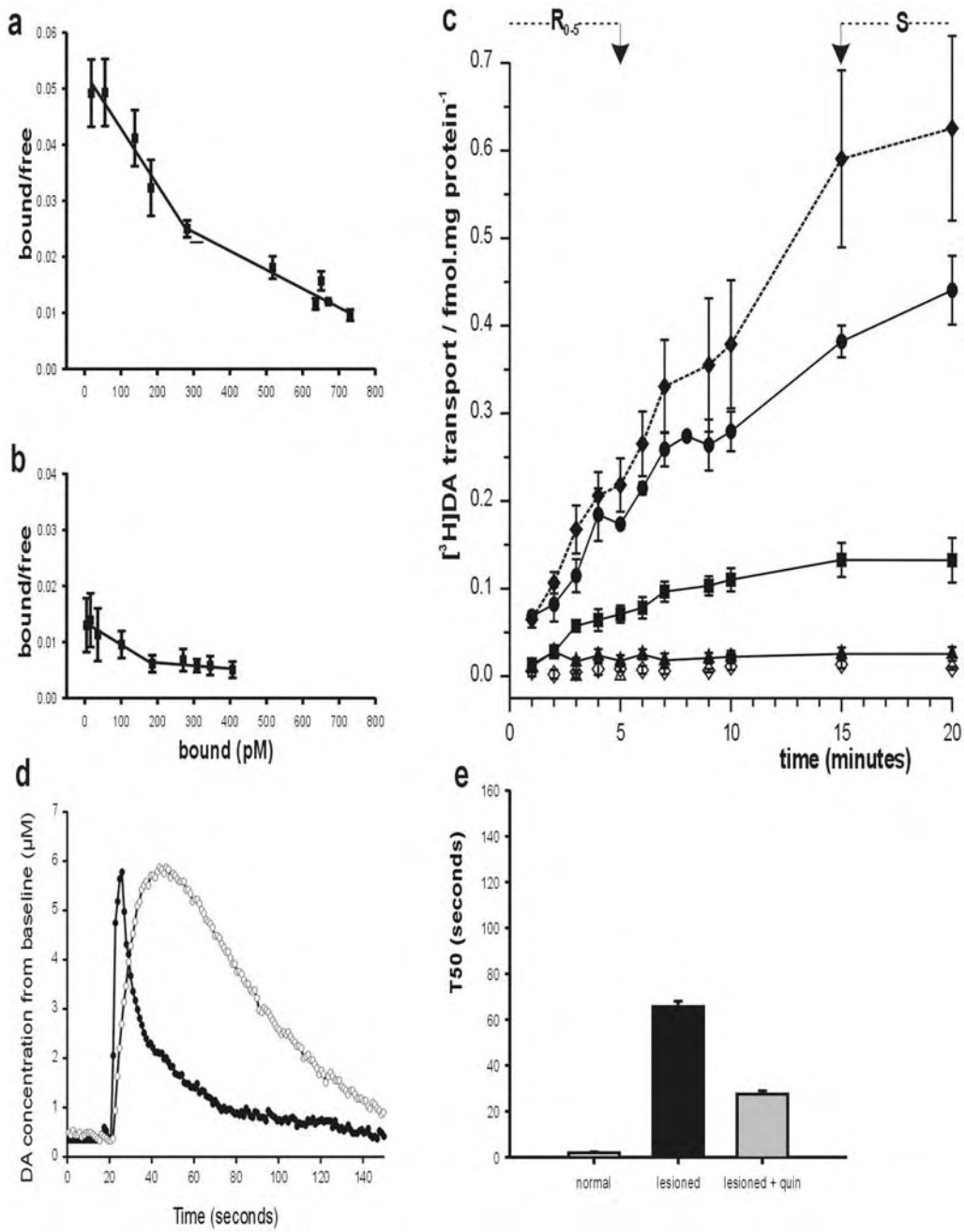

Fig. 3. These figures demonstrate that regenerated terminals a have dopamine transport with reduced velocity and affinity. However the D_2R appears to be normally responsive to quinpirole suggesting that it has normal function. (a and b) Scatchard plots of [^{3}H]Mazindol binding to the DAT in the dorsal striatum. (a) normal animals and (b) lesioned animals. Data from normal animals required a two-line fit indicative of two distinct binding sites, one of high affinity and a second of lower affinity. Following partial SNpc lesions, the scatchard plots also required a two-line fit. Although the Kd of high affinity sites in lesioned and unlesioned animals were similar, density was reduced by almost 40% in lesioned animals. In contrast, the low affinity site had a very high

earlier, the D_1R may have a specific role in cell division (Ohtani et al., 2003), with D_1R activation being critical for progenitor cells contained within the lateral ganglionic eminence progressing from G_1 to S phase of the cell cycle.

In conclusion, the origin of TH^{-ve} neurons observed in the SNpc must remain a matter of speculation. They are likely to be a fruitful area for further study, providing insights into the process of neuronal repair in the brain, as well as for the deployment of stem cells for the repair of the nigra.

2.5. DO SPROUTED TERMINALS FUNCTION NORMALLY?

If terminal density is regulated so as to maintain appropriate dopamine levels in the synaptic cleft (Parish et al., 2001), it implies that mechanisms for release and transport of dopamine on these newly formed terminals are amenable to regulation. In normal nigrostriatal terminals, dopamine synthesis and release is highly regulated. Presynaptic D_2R inhibit nerve terminal excitability (Bunney et al., 1973; Tepper et al., 1984) and reduces dopamine release (Ungerstedt et al., 1982; Bowyer and Weiner, 1987), partially mediated via activation of potassium channels (Lacey et al., 1987; Cass and Zahniser, 1990). Activation of D_2R by dopamine reduces cAMP production and thereby reduces dopamine synthesis by AC-dependent phosphorylation of TH, the rate limiting enzyme in the dopamine synthesis pathway (el Mestikawy et al., 1986; Onali et al., 1988; Lindgren et al., 2001). The D_2 autoreceptor is also tightly linked to DAT, both anatomically (Hersch et al., 1997) and functionally (Kimmel et al., 2001; Robinson, 2002). Thus, the return of regulated function of dopamine terminals following injury might be expected to include evidence of coordinated D_2R and DAT interaction as evidenced by regulated dopamine release and turnover.

Most, if not all, dopamine terminals in the striatum are newly formed when the CPu is re-innervated following an SNpc lesion and the ultrastructure of these terminals is altered, suggesting they may produce, store and release more dopamine than normal terminals (Finkelstein et al., 2000; Stanic et al., 2003a). These changes include increased terminal

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density. (c) [^{3}H]dopamine ([^{3}H]DA) transport into synaptosomes. The rate of dopamine transport over the first five minutes (R0-5) and the saturation concentration (S), was calculated. In the normal animal (black diamond), and those with small lesions (black circles), a transient drop in the rate or "notch" occurred between 7 and 10 min, suggesting a point of transition to a second lower affinity transporter that continued transporting till about 15 min. Mazindol (white diamond) reduced the rate of transport. In small lesions (0–30%, filled circle), R0-5 was near normal, but S was reduced to almost half of normal. Following medium sized lesions (black squares), both R0-5 and S were significantly reduced. In large lesions ($>70\%$, black triangles), both R0-5 and S were greatly reduced. Mazindol reduce both R0-5 and S in all lesioned animals (white symbols). (d) Dopamine concentration in the dorsal striatum of normal and rats lesioned for 16 weeks, made before and after local application of 325 ± 70 nl of 200 μM dopamine in the vicinity of carbon-fibre recording electrodes. In normal animals (black circles), dopamine concentration rises rapidly to a peak and is also cleared promptly. Following a lesion (white circles), the time to peak dopamine concentration is significantly longer and clearance is greatly prolonged. (e) In vitro recordings showing dopamine uptake in synaptosome preparations from the dorsal striatum of normal rats and those lesioned for 16 weeks, and the effects of quinpirole. This shows differences in the rate of dopamine uptake after addition of 12 μl 0.25 mM dopamine to striatal synaptosomes from normal and lesioned animals and synaptosomes from lesioned animals that were pre-treated with quinpirole. Figures from Stanic et al. (2003b).

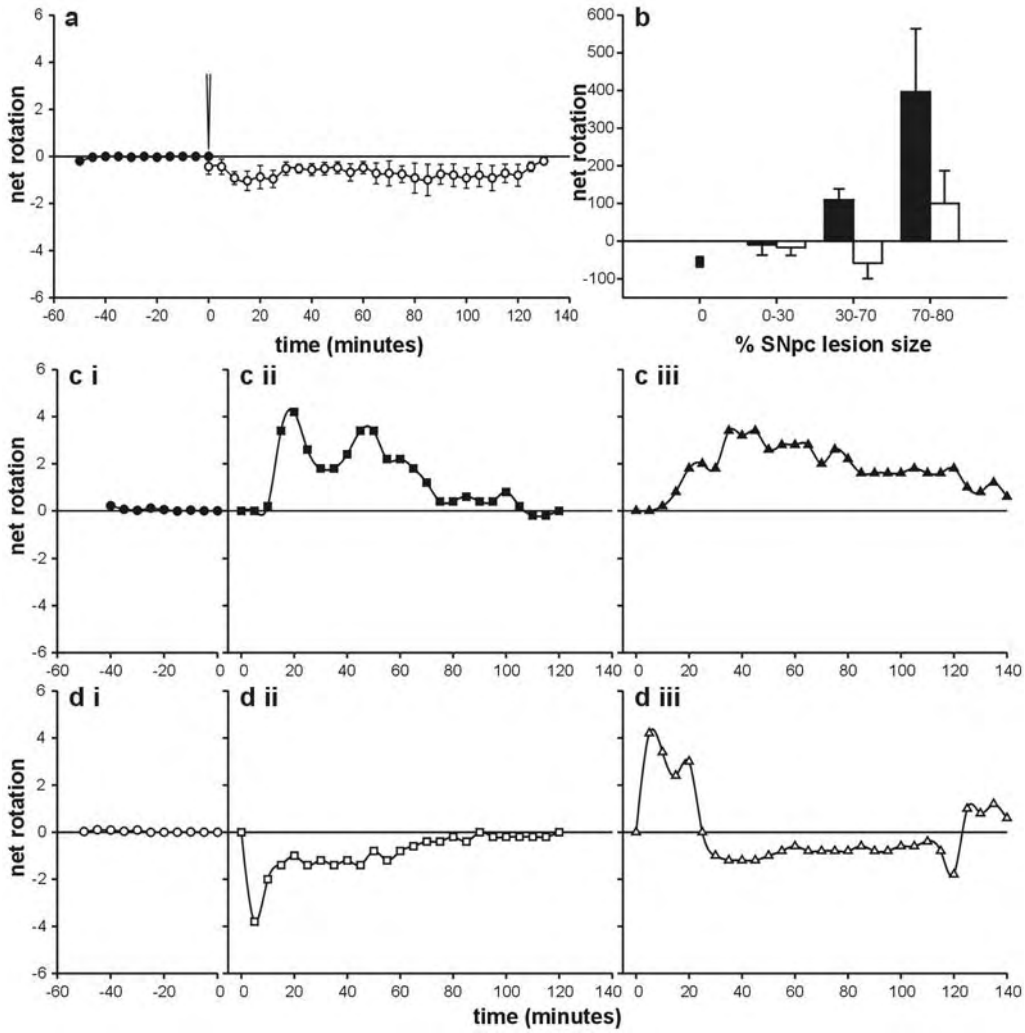

Fig. 4. Rotational behavior of individual animals in response to administration of amphetamine (5 mg kg^{-1} i.p.). In these graphs, each symbol represents the net rotation (right turns minus left turns) made in a 5 min period divided by 5 to obtain the average number of turns per minute in that interval. (a) Turning behavior of a normal animal. Black circles, behavior before amphetamine; White circles, behavior after amphetamine; V, amphetamine injection. (b) From the plot of each animal's rotational behavior, an estimate of the area under the curve was made by adding each data point for 140 min after amphetamine administration. Animals were grouped according to lesion size and the mean area (±SE) for each group was plotted. The small black square shows the normal unlesioned animals rate of turns to the left following amphetamine. The black bars are from animals 4 weeks postlesion and white bars are from animals 16 weeks postlesion. At 4 weeks, lesion size was proportional to the extent of right turning bias but by 16 weeks, animals whose lesions were less than 70% had a near normal propensity to turn to the left. Animals with lesions larger than 70% still showed a right ward bias, but this was much less marked than in the 4 week animals. (c) Behavior of animals 4 weeks after a lesion. (c i) Averaged response of all animals prior to amphetamine administration ($n = 15$). (c ii) Response of an animal with a 40% lesion. (c iii) Turning response of an animal with a 68% lesion. (d) Behavior of animals 16 weeks after a lesion. (d i) Averaged response of all animals prior to amphetamine administration ($n = 12$). (d ii) Response of an animal with a 44% lesion. (d iii) Turning response of an animal with a 65% lesion. In the absence of amphetamine, animals did not tend to turn in either direction (panel (a), (c i) and (d i)) although amphetamine treatment in normal animals induced a persistent but modest bias toward leftward rotation that persisted for 2 h after injection

size, increased number of vesicles, contacts onto more proximal targets and increased numbers of mitochondria; changes that should result in increased synaptic efficiency and therefore constitute an appropriate compensatory response to injury. Transport of dopamine into these newly formed terminals is reduced (Fig. 3), presumably due to a substantial increase in the density of the low affinity transporter sites (Stanic et al., 2003b) resulting in an abnormal uptake of dopamine and doubling of the time required to clear released dopamine from the synaptic cleft. It is likely that the turnover and functionality of DAT is regulated through D_2 autoreceptors (Hersch et al., 1995; Kimmel et al., 2001; Robinson, 2002). Normal synaptosomes exposed to quinpirole demonstrated that activation of D_2R increases uptake of dopamine (presumably through the transporter). A similar elevation is seen in synaptosome preparations from lesioned animals (as demonstrated by a reduction in the time taken to clear dopamine from the synapse; Fig. 3) suggesting that the D_2R/DAT molecular interaction is preserved in new synapses.

Interestingly, release of dopamine is normal after lesioning as measured by the peak dopamine concentration produced by KCl injection (Fig. 3) (Stanic et al., 2003b). The electronmicroscopic appearance of postlesion terminals with the greater number and larger size of vesicles would intuitively suggest that these terminals are capable of delivering larger amounts of dopamine into the cleft. Although larger vesicle numbers and size suggest increased capacity for dopamine release, it may also reflect increased demand for synthesis in lieu of the impaired transport. Although the peak dopamine concentration obtained is comparable in lesioned animals, the time to reach the peak is significantly longer, suggesting that the rate of release in lesioned animals is less than normal (Garris et al., 1997). However, other studies have found that release of dopamine in the partially denervated striatum was similar to that in the intact striatum (Robinson and Whishaw, 1988).

The extent of SNpc lesions, and by implication the extent of CPu dopamine innervation is frequently assessed by examining the animal's rotational response to direct or indirect dopamine receptor agonists (Fig. 4) (Ungerstedt, 1968; Ungerstedt and Arbuthnott, 1970; Pycock, 1980; Perese et al., 1989; Thomas et al., 1994; Hansen et al., 1995; Wenning et al., 1996; Yurek, 1997). In the case of partial lesions, lesion size, measured by the number of remaining TH-IR neurons, dopamine cell loss was usually greater than 90% in rotating animals (Perese et al., 1989; Ichitani et al., 1991; Sakai and Gash, 1994; Hansen et al., 1995; Blanchard et al., 1996; Brecknell et al., 1996) suggesting that compensatory processes are active to maintain normal motor function until the lesions is almost complete. Following amphetamine administration normal animals demonstrate a propensity to rotate left (Jerussi and Glick, 1974; Pycock, 1980). Four weeks after lesioning, amphetamine induces turning toward the side of lesion (Ungerstedt and Arbuthnott, 1970; Pycock, 1980; Dravid et al., 1984; Stanic et al., 2003b), with this effect being proportional to lesion size. At 16 weeks, by which time animals with small and medium lesions ($<70\%$) have established a normal density of terminals in the striatum, the pattern of turning substantially alters (Stanic et al., 2003a,b).

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(panel a). Panel (c ii) shows that even with moderate lesions animals tended to turn toward the right whereas by 16 weeks turning behavior had tended toward the left, even after large lesions. Nevertheless rotational responses were often complex at 16 weeks (d iii). On the y-axis, positive numbers indicate right turns and negative numbers indicate left turns. Figures from Stanic et al. (2003b).

Most animals with small lesions and many with intermediate lesions turned left or have only a modest tendency to turn toward the side of the lesion (right side). Only animals with large lesions persist in turning toward the lesioned side. Thus amphetamine induced turning provides a functional measure of the degree to which regenerated dopamine terminals can release dopamine. Amphetamine induced rotation is therefore a better measure of the degree of functional reinnervation rather than the size of a lesion.

As synaptic contacts are being reestablished, it is likely that the high-affinity DAT is down regulated to maintain dopamine concentrations in the synaptic cleft. With time, and as the number of contacts normalize, normal transport may also be restored. This however, requires a lengthy process and would not be completed in animals with extensive lesions, even after 16 weeks. Blanchard et al. (1996) observed growth cones entering the striatum seven months after partial lesions, suggesting that 12 months or more may be required for normalization of synaptic function (Blanchard et al., 1996).

2.6. FUNCTIONAL IMPLICATIONS OF SPROUTING

The implications of sprouting of the dopamine neurons as models for the repair of the nervous system are evident. Similarly, the importance of an understanding of the factors that contribute to and regulate the phenotype of repairing neurons is also clear. This information will be essential in understanding how neurons may participate in both plasticity and repair as well as in the deployment of transplanted neural stem cells.

It is however interesting to speculate further on the implications these findings may have for Parkinson's disease and drug-induced dyskinesia. The fact that symptoms of Parkinson's disease do not become apparent until some 60–80% of neurons are lost does suggest a compensatory response. Sprouting of presynaptic neurons may indeed be a significant factor in this compensation. Stanic et al. (2003b) described complex patterns of turning in response to amphetamine in animals in whom reinnervation has occurred. These patterns are reminiscent of peak dose and biphasic dyskinesia of Parkinson's disease (Poewe, 1993). It is conceivable that dysregulated terminals with prolonged reuptake of dopamine from arbors that stretch throughout much of the striatum could result in complex patterns of dopamine release. Stanic et al. (2003b) speculated that sprouting of axons, whether drug induced (by D_2R antagonists like haloperidol) or as a response to lesioning, will result in abnormal dopamine delivery. This abnormal delivery will be to unusually large regions as a consequence of both the large terminal arbors of individual axons and because of impaired synaptic clearance and reduced function of DAT. These factors and the altered synaptic contacts form a common basis for the dyskinesia of Parkinson's disease, tardive dyskinesia and possibly the dyskinesia that follows transplantation therapy (Freed et al., 2001). The altered uptake is likely to lead to more prolonged stimulation of postsynaptic receptors with altered, even augmented patterns of postsynaptic activation leading to altered patterns of motor activation. As previously noted, nigrostriatal synaptic terminals most commonly form contacts with dendritic spines and shafts, and less commonly with the somata of striatal neurons (Freund et al., 1984; Zahm, 1992; Groves et al., 1994; Anglade et al., 1996; Descarries et al., 1996; Hanley and Bolam, 1997; Ingham et al., 1998). Following lesioning, the number of distal dendrite and spine contacts decrease and consequently there is a greater proportion of more proximal dendrite and direct contacts with the soma (Ingham et al., 1996; Ingham et al., 1998;

Stanic et al., 2003b). Recently, Reynolds et al. (2001) described how stimulation of the SNpc induced potentiation of the glutamatergic synapses between the cortex and the striatum that was dependent on the activation of dopamine receptors (Reynolds et al., 2001). The cortico-striatal glutamatergic fibers synapse onto the ends of dendritic spines of the striatal neurons whereas the SNpc terminals normally synapse onto the shaft. As more proximal synapses are believed to elicit greater physiological changes in the target neurons than distal synapses (Pickel et al., 1992), the more proximal site of termination of the reinnervated dopamine terminals could enhance the efficiency of dopamine augmentation of glutamatergic transmission. Indeed, Picconi et al. (2002) described that plasticity at the cortical projection onto spiny neurons was altered by selective dopamine receptor blockade and following dopamine denervation but restored by L-DOPA therapy (Calabresi et al., 2000; Centonze et al., 2001; Picconi et al., 2002). Following chronic neuroleptic drug treatment, there is persistent alteration in dendrites and spines, especially in the ventral striatum. As lesioning and haloperidol therapy both produce sprouting (Parish et al., 2001), it is possible that this sprouting provides the drive for the synaptic remodeling described here and elsewhere (Meshul and Tan, 1994; Meredith et al., 2000; Meshul and Allen, 2000).

The notion that the strength of the glutamatergic cortico-striatal synapse is modified by dopaminergic influences has been difficult to confirm directly, but has a body of evidence to support it (Hyman and Malenka, 2001). Cocaine can elicit changes in the relative ratios of NMDA and AMPA receptors (Thomas et al., 2001) and rewards can produce potentiation of cortico-striatal synapses when dopamine is released in response to those rewards (Reynolds et al., 2001). Long term potentiation at the cortico-striatal synapse appears dependent on dopamine because it is suppressed by D_1R blockade, and cannot be elicited in mice with lesioned SNpc (Centonze et al., 2001). D_1R activation depolarizes spiny neurons and promotes their vigorous spiking by enhancing L-type Ca^{++} currents (Nicola et al., 2000). While this will result in diminished sensitivity of the spiny neurons to weak, transitory cortical inputs, it will enhance their response to strong, maintained cortical synaptic inputs (Hernandez-Lopez et al., 1997). Whether this reflects D_1R or D_5R involvement is unclear from these experiments as D_1R blockade cannot discriminate between these dopamine receptor subtypes. On the surface it appears that the D_5R may be involved as experiments in $D_1R(-/-)$ mice have shown that mice lacking the D_1R develop place preference in response to cocaine as do wt controls (Miner et al., 1995).

3. SUMMARY

Advances in molecular biology have resulted in a number of mutant mice with defined genetic defects at dopamine receptor loci. The data relating to the analysis of the knockout phenotype, both with respect to baseline parameters and in response to drug administration is currently available for all five dopamine receptors as well as for a number of double knockout mice both within the D_2-like subfamily and between the D_1- and D_2-like subfamilies. Despite a large number of documented developmental compensatory changes in knockout mice, major advances have been made in understanding the role of these receptors in the regulation of reward processes, in the control of dopaminergic neurotransmission and more recently in the modulation of sprouting after neurotoxic injury to substantia nigra. The D_2R autoreceptor appears to have a pivotal role in regulating sprouting of dopaminergic neurons in the adult brain after injury. Future animal

models will likely avoid developmental compensation by the use of inducible deletion paradigms. Finally, further research efforts will also identify the exact downstream second messenger systems mediating dopamine stimulated neural processes in the normal brain, following injury and in response to acute and chronic drug administration.

4. ABBREVIATIONS

AC	adenylate cyclase
cAMP	adenosine 3′,5′-cyclic monophosphate
CPu	caudate putamen
DARRP-32	dopamine- and cAMP-regulated phosphoprotein
DAT	dopamine transporter
ES	embryonic stem
GABA	gamma-amino-butyric acid
GAD	glutamic acid decarboxylase
GDP	guanosine diphosphate
IR	immunoreactive
MFB	medial forebrain bundle
MPTP	methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAcc	nucleus accumbens
PI	phosphatidylinositol
PLC	phospholipase C
PLD	phosholipase D
PPI	prepulse inhibition
PTX	pertussis toxin
SN	substantia nigra
SNpc	substantia nigra pars compacta
TH	tyrosine hydroxylase
VTA	ventral tegmental area
Wt	wild type
6-OHDA	6-hydroxydopamine

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CHAPTER IV

Structural and functional interactions in the striatum at the receptor level

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ABSTRACT

Dopamine is a neurotransmitter which has defied interpretation in part because of the multiplicity of its actions. In this chapter we focus on dopamine's actions in the striatum. We review evidence that the dopamine signal is time-specific but not spatially focused at the synaptic level. However, the distribution of different dopamine receptor subtypes may mean that naturally-released dopamine has pathway-specific actions. The actions of dopamine include, effects on glutamate receptors on nearby synapses, and modulation of postsynaptic ion channels. Both kinds of dopamine effects may be strongly dependent on prior membrane potential activity or on recent presynaptic activity. The effects of dopamine may also be divided into those immediate and reversible effects that occur in the presence of the agonist, and more persistent effects including both functional and structural synaptic plasticity. We suggest that the more immediate and reversible actions of dopamine are linked to initiation of movements, brought about by facilitation of striatal output by anticipatory firing of dopamine cells in response to incentive cues. The longer-lasting actions of dopamine may underlie the reward-related learning, by potentiation of corticostriatal synapses. This provides a framework for the coordinated action of dopamine in natural behavior. Both these dopamine effects are compromised by perturbations of the dopamine system as they occur in neurological disease, or as a consequence of dopaminergic drugs.

1. CONTEXT

One of the major problems in thinking about dopamine neurotransmission is the number of features which do not fit into the textbook model of classical synaptic transmission, based on the neuromuscular junction. These include an extremely wide divergence of release sites of a single axon, extrasynaptic location of receptors, termination of neurotransmitter action by diffusion and uptake from extrasynaptic sites, and significant overflow of neurotransmitter from the synaptic cleft into the extracellular space.

In the recent decades, dopamine has emerged as a key, through somewhat controversial, neurotransmitter in incentive motivation and reinforcement learning. There is a need to integrate the detailed biophysics of dopamine function with the requirements for

the behavioral mechanism. The spatial and the temporal resolution of the dopamine signal is of fundamental importance for this integration.

In this chapter, we undertake a review of the quantitative aspects of dopamine neurotransmission of relevance to the spatial and temporal specificity of the dopamine signal. The kinetics of dopamine release and clearance are considered in order to estimate the temporal and spatial concentration distribution of dopamine. The location of receptors and their sensitivity to dopamine are taken into account. We then consider the regulation of ion channels by the G proteins activated by dopamine, and how this might explain the effects of dopamine on the whole cell. Finally, we consider the regulation by dopamine of corticostriatal inputs to the spiny cells.

Together, the evidence reviewed suggests that dopamine acts diffusely, but rapidly, to modulate the responsiveness of spiny neurons to motivationally significant stimuli, to facilitate long-lasting changes in synaptic efficacy, and to maintain the physical structure of corticostriatal synapses.

2. THE NATURE OF THE DOPAMINE SIGNAL

Historically, varicosities have been thought to be the site of synaptic contact of dopamine axons in the striatum. The points of actual synaptic contact are so small that they are difficult to detect in a single thin electron microscopy (EM) sections: serial sections are needed and synaptic specializations may only be present in one section. Studies using the serial EM have revealed that varicosities are not preferentially involved in synaptic contacts (Pickel et al., 1982; Freund et al., 1984; Groves et al., 1994). Counts of varicosities, therefore, do not necessarily reflect the dopamine synapse numbers: it is necessary to use dopamine specific labels, such as antibodies to tyrosine hydroxylase (TH) or transporter-specific markers, such as 5-hydroxy-dopamine (5-OHDA) together with serial EM.

A common feature of the dopamine synapse is its termination on the necks of spines that also receive an asymmetrical synapse on the head (see Fig. 1A). There is good agreement that just over half of the structures that are postsynaptic to dopaminergic synapses are on spines. Freund et al. (1984) reported that 56.5% of all TH-immunoreactive synapses were on spines, and Groves (1994) reported 56% of 5-OHDA-labeled synapses were on spine necks or heads. In every case of a dopaminergic synapse on a spine, a corresponding asymmetrical synapse has been identified on the same spine. The asymmetrical synapses is presumed to be of cortical or thalamic origin. This arrangement of a spine head asymmetrical synapses with a dopaminergic synapse at the spine base, has given rise to the view that dopamine is involved in the regulation of current flow from the spine head to the dendrite.

However, not every asymmetrical synapse has a dopamine input onto the same spine. The highest estimates of the fraction of spines that are innervated by dopamine come from Freund et al. (1984) who found that 39% of spines on reconstructed dendrites of a single striatonigral cell received one asymmetrical and one TH-positive symmetrical synapse. In contrast, estimates based on quantitative neuroanatomy, together with some assumptions about the distribution of synapses in the striatal volume give a much smaller fraction. Table 1 summarizes these estimates.

As presented in Table 1, the density of dopamine synapses in the striatum can be estimated from the density of all synapses (Ingham et al., 1998) by multiplying with the

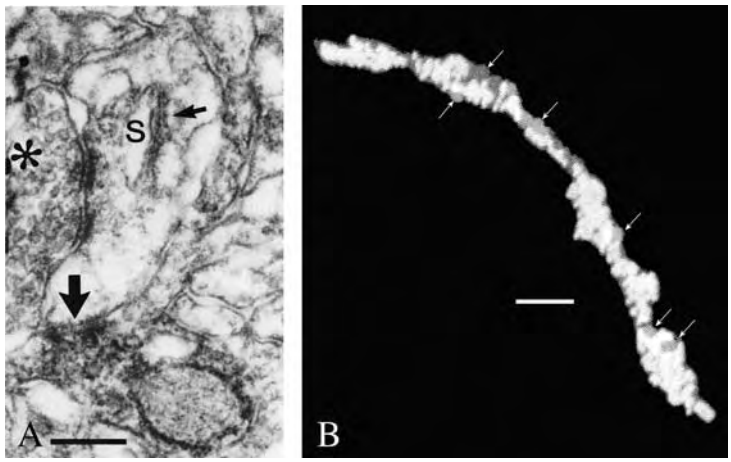

Fig. 1. A. Morphology of dopamine synapses. Electron micrograph of neostriatal section showing a TH-positive bouton in symmetrical synaptic contact (arrow) with a dendritic spine (S) which receives an asymmetrical synapse on its head from a bouton containing small round vesicles (asterisk); spine apparatus (small arrow). Scale bar 0.2 μm. From Fig. 2F of Freund et al. (1984), with permission. B. Three-dimensional reconstruction of a dopaminergic axon found in a series of 70 sections of the neostriatum, showing the distribution of synaptic sites (arrows). Scale bar 1.0 μm. Modified from Fig. 3C of Groves et al. (1994), with permission.

TABLE 1. *Quantitative aspects of the dopamine system*

	Quantity	Value	Ref.
i	Density of symmetric synapses in striatum (μm^{-3})	0.235	1
ii	Density of asymmetric synapses in striatum (μm^{-3})	0.915	1
iii	Density of all synapses (μm^{-3})	1.15	i, ii
iv	Fraction of all synapses in striatum that are dopaminergic	0.09	2
v	Density of dopamine synapses (μm^{-3})	0.104	iii, iv
vi	Average nearest-neighbor distance between dopamine synapses (μm)	1.18	v, 3
vii	Density of striatal cells (mm^{-3})	108,469	4
viii	Number of dopamine synapses per striatal cell	954	v, vii
ix	Number of asymmetric synapses per striatal cell	8436	ii, vii
x	Ratio of striatal asymmetric-to-dopamine synapses	8.84	viii, ix
xi	Total number of cells in pars compacta	7200	5
xii	Total number of cells in striatum	2.791×10^6	5
xiii	Total number of dopamine synapses in striatum	2.7×10^9	xiii, viii
xiv	Number of striatal dopamine synapses per pars compacta cell	369,881	xiii, xi
xv	Volume of dopamine cell arborization (mm^3)	1	6
xvi	Average nearest-neighbor distance for dopamine synapses of single cell (μm)	7.72×10^{-6}	xv, 3

References: (1) Ingham et al. (1998); (2) Groves et al. (1994); (3) Clark and Evans (1954, 1979); (4) Arbuthnott et al. (2000); (5) Oorschot (1996); (6) Prensa and Parent (2001). Notes (i–xvi) refer to corresponding lines of this table.

fraction that appear to be dopaminergic, based on their morphology in serial EM sections (Groves et al., 1994). Dividing this number by the density of striatal cells shows there are on average 954 dopamine synapses per striatal cell. As noted above, there is good agreement that about 56% of these terminate on spines (Freund et al., 1984; Groves et al., 1994). Taking this into account suggests that there are 534 dopamine-spine synapses per striatal cell. The total number of asymmetric synapses per striatal cell is similarly estimated to be 8436 (Table 1), of which 95% terminate on spines or profiles similar to spines (Ingham et al., 1998). Thus, by this argument only about 7% of spines receive both dopamine and corticostriatal synapses. This inconsistency with the value obtained from a single striatonigral cell (Freund et al., 1984) suggests that the 39% figure is not representative and, on average, a much smaller proportion of corticostriatal synapses has a dopamine input onto the same spine.

At present, it is difficult to reconcile these two different pieces of evidence unless there is a highly nonuniform distribution of dopamine synapses. There may be a bias towards dopaminergic synapses on striatonigral neurons, as suggested by the results of Freund et al. (1984). However, for this explanation to be consistent with the numbers given above would require that dopamine terminals synapse exclusively with striatonigral neurons. This seems unlikely to be the case as Sesack and others have shown striatal neurons exhibiting D2 labeling (and hence, probably not striatonigral neurons) were contacted by dopamine terminals immunoreactive for TH (Pickel et al., 1982; Sesack et al., 1994). On the other hand, a spatial nonuniformity of some sort seems likely: Groves et al. (1995) wrote that 'Dopaminergic synaptic contacts appeared to be distributed nonuniformly, even within a relatively small volume of neuropil.... Labeled axons appeared to form many synapses exclusively in one region, but none as they traversed an adjacent area that also seemed to contain potential synaptic sites'.

Does the foregoing mean that dopamine acts on only 7% of corticostriatal synapses? If only a small minority of corticostriatal synapses have a dopamine input onto the same spine, does this in turn imply that dopamine acts only at a selected subset of spines? Alternatively, how much importance should be attached to the location of dopamine synapses on the same spine, as opposed to neighboring synapses on different spines, given that there is significant overflow and diffusion of dopamine from the synaptic cleft? To address these issues, precise quantification of several aspects of dopaminergic signaling is required, including: (i) the spatial relationship between the dopamine release sites and the receptors; (ii) the spatiotemporal distribution of dopamine produced by the interaction of the release, the diffusion and the reuptake of dopamine; (iii) the release of dopamine brought about by the actual firing pattern of dopamine neurons in different behavioral contexts; and (iv) the associated affinities and potencies of target receptors. These will be covered in the next four sections.

2.1. SPATIAL RELATIONSHIP BETWEEN DOPAMINE RELEASE SITES AND RECEPTORS

The definition of a synapse requires at least the presence of a presynaptic element containing a concentration of vesicles and an apposed postsynaptic element, from which it is separated by the synaptic cleft (Peters et al., 1991). The vesicles of the dopamine synapses are concentrated around the points of synaptic contact rather than around the mitochondria that are part of the varicosities, so the release is probably punctate from the synaptic active zones. Notwithstanding the existence of synaptic specializations and

vesicles at presumed release sites, it is also important to know whether the released dopamine is confined within the synaptic cleft or, alternatively, the release site is the center of a sphere of influence, the extent of which may be regulated by other things. Evidence reviewed supports the idea of a sphere of influence over which dopamine acts on receptors outside the synaptic cleft, and diffuses to many adjacent asymmetrical synapses. Two important factors are, therefore, the distance between the dopamine release sites and the targets, and the diffusion distance of effective concentrations of dopamine.

2.1.1. Distance between release sites

What is the average distance between a dopamine terminal and its nearest asymmetric terminal? This is calculated in Table 1, based on the available quantitative neuroanatomy. From the density of synapses in the striatum determined by unbiased stereology (Ingham et al., 1998), the density of dopamine synapses was estimated by applying the proportion reported by Groves et al. (1994). We acknowledge that this proportion, namely 9%, is somewhat tentative as it is based on the analysis of small blocks of tissue. However, it is the best available at present. Multiplying the density by this proportion gives an average density of the dopamine synapses of $0.105\,\mu m^{-3}$. The average nearest-neighbor distance between dopamine synapses is calculated from this density using a formula which assumes a uniform, random distribution of terminals in the striatal volume (Clark and Evans, 1954, 1979). This gives an average nearest-neighbor distance between dopamine synapses of about 1.2 μm. Thus, despite the high ratio of asymmetrical to dopaminergic synapses, which is in the order of 9:1 (Table 1), the majority of corticostriatal synapses lie within a short range, less than 1.2 μm, of a dopamine terminal. Although we consider this estimate realistic, it should be emphasized that a critical value in the calculation is the proportion of all synapses that are dopaminergic, is based on the analysis of small blocks of tissue (Groves et al., 1994).

An alternative estimate of the density of dopamine terminals could, in principle, be obtained from counting varicosities and correcting for the fractions of varicosities without synapses, and the fraction of synapses not on varicosities. Doucet et al. (1986) estimated an average density of dopamine varicosities of $0.1\,\mu m^{-3}$. This number is remarkably close to the density of synapses estimated above.

Yet, varicosities do not equal synapses, and several authors have described dopaminergic varicosities without any synaptic specializations, and vice versa. This matter is somewhat controversial. Smith et al. (1994) noted that studies which report a large proportion of nonsynaptic relationships were carried out in single sections (Arluison et al., 1984; Triarhou et al., 1988; Zahm, 1992). In contrast, the majority of boutons are seen to form symmetric contacts in serial sections (Pickel et al., 1981; Freund et al., 1984; Smith et al., 1994). However, Groves (1994, 1995) using 3D serial reconstruction techniques showed the locations of synapses are not correlated with dilated portions of the axon (see Fig. 1B). Similarly, Descarries (1996) using the EM histochemistry and the EM autoradiography showed that only 30–40% of the dopamine varicosities formed symmetrical synapses. Conversely, many synapses – possibly twice as many – are located in the nonvaricose segments of the axon (Groves et al., 1994, 1995). A similar proportion has been determined in the dopamine synapses of the monkey prefrontal cortex (Smiley and Goldman-Rakic, 1993). Thus, although a large fraction of varicosities lack synapses, there is a correspondingly large fraction of synapses not on varicosities. Therefore, the estimated density of dopamine synapses is, by coincidence, numerically

similar to the density of varicosities. This value is in remarkable agreement with the estimates made above, which used independent data.

Thus two independent estimates of the density of dopamine synapses are in close agreement. This increases confidence in the estimate of the average distance between the nearest neighbors, namely 1.2 μm. What does this mean in the context of the striatal neuropil? Figure 1A shows the dimensions of spines, dendrites and axons involved in dopaminergic synapses. It is clear from these dimensions that dopamine terminals on the spine necks are on the order of 0.2 μm away from the asymmetrical synapses. This distance is nearer than the average nearest-neighbor distance, and hence may represent a functional specialization. Figure 2 shows a schematic representation of the packing arrangement of the synaptic structures in the striatal neuropil, which gives an appreciation of the proportions of different structures and the near-neighbor relations of dopamine terminals and targets. The ability of the dopamine to act on the surrounding targets over dimensions of 1.2 μm, depends also on the ultrastructural localization of the receptors and the spatiotemporal distribution of dopamine after its release.

2.1.2. Subcellular localization of dopamine receptors

The physiological effects of dopamine transmission in the brain are mediated by a family of G-protein coupled receptors. Kebabian and Calne (1979) proposed two classes of dopamine receptor, D1 and D2, based on cAMP assays and ligand binding. These have different biochemical and pharmacological properties and physiological functions. Selective agonists and antagonists exist for each of the two subtypes. Different G-proteins and effectors are involved in the signaling pathways of the D1 and the D2 subtypes.

Five pharmacologically distinct dopamine receptors have been identified by the molecular cloning techniques. These have been grouped into D1-like (D1 and D5) and D2-like (D2, 3 and 4) receptors on the basis of their pharmacological profiles and sequence (Sibley and Monsma, 1992). There may be other subtypes yet to be discovered. Localization of the receptor subtypes using specific antibodies is a direct means of studying receptor expression, which can be combined with the EM to provide ultrastructural localization. Several laboratories have made antibodies against specific peptides from dopamine receptors and used them for EM immunohistochemistry. With these methods, it has become apparent that dopamine receptors are not concentrated immediately within the dopaminergic synapse but are located some distance away, sometimes in association with other types of synapses (Levey et al., 1993; Hersch et al., 1995; Yung et al., 1995; Caille et al., 1996). The following sections review the pre- and postsynaptic localization of dopamine receptors.

2.1.3. Dopamine receptor labeling in terminals presynaptic to asymmetrical synapses

Examination of dopamine receptor labeling in terminals that are presynaptic to asymmetrical synapses (which include corticostriatal terminals) has produced variable results. Several authors observed D1 labeling in axon terminals, which formed asymmetric synapses with dendritic spines (Huang et al., 1992; Bergson et al., 1995; Yung et al., 1995). Such labeling is not common and other authors report D1-labeled terminals as occurring exceedingly rare (Hersch et al., 1995) or not at all (Levey et al., 1993).

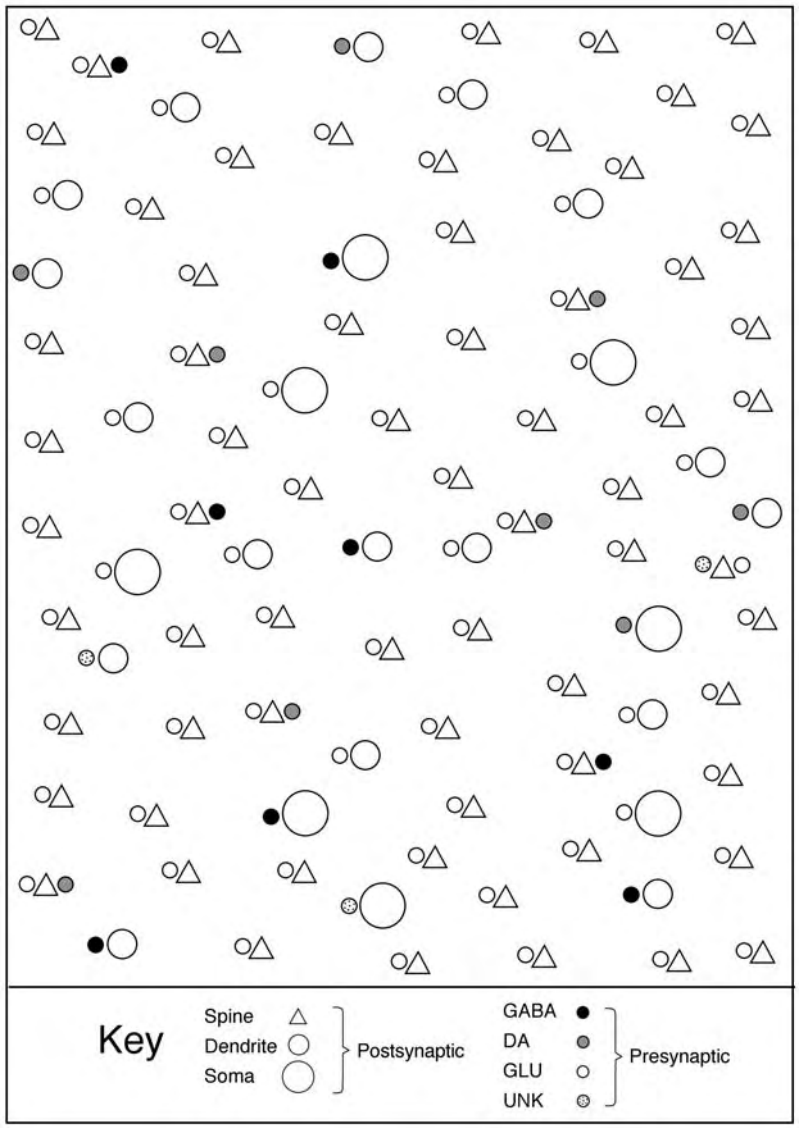

Fig. 2. Relative abundance and close packing of different postsynaptic targets of dopaminergic synapses in the striatum. Note the termination of a dopamine (DA) and a glutamate (GLU) presynaptic terminal on the same postsynaptic spine in a minority of cases. Note also that the majority of glutamate synapses are within one or two synapses of a dopamine terminal. GABAergic (GABA) and unknown (UNK) terminals are also shown.

A similar picture applies to D2 labeling. Generally, a small number of D2 immunoreactive terminals forming asymmetrical synapses have been observed (Sesack et al., 1994; Hersch et al., 1995; Yung et al., 1995), or none at all (Levey et al., 1993). Unfortunately there has been no quantification of the fraction of such terminals which are labeled. However, as for the D1 receptors, their qualitative descriptions suggest only a small fraction of corticostriatal terminals to be D2 positive.

2.1.4. Dopamine receptor labeling in terminals presynaptic to symmetrical synapses

Hersch et al. (1995) found that D1 immunoreactive terminals presynaptic to symmetrical synapses were exceedingly rare whereas the D2 immunoreactive terminals were quite frequent. Synapses formed by D2 immunoreactive terminals were not easy to identify due to a lack of pronounced pre or postsynaptic densities, but many D2 positive presynaptic terminals made symmetrical synapses with dendritic shafts and spines. Consistent with this, many presynaptic D2 receptors were also seen in terminals which were not positive for TH, suggesting they may be heteroreceptors (Sesack et al., 1994). This is confirmed by the demonstration of the D2 positive GABA axon terminals presynaptic to symmetrical synapses (Delle Donne et al., 1997).

Levey et al. (1993) found that axon terminals immunoreactive for D1 and D2 receptor proteins formed symmetrical synapses exclusively, and primarily with unlabeled dendritic shafts. In cultures, D1 and D2 receptors have been colocalized to terminals of intrinsic neurons (Wong et al., 1999). Functional D1 receptors have also been demonstrated on the terminals of striatal cells in the substantia nigra (Fiorillo and Williams, 1998). Collectively, the results for D1 and D2 receptors suggest their presence on the terminals of intrinsic GABA neurons.

Consistent with pharmacological evidence, many D2 receptors are located presynaptically on dopaminergic terminals. Sesack et al. (1994) found that some D2 was colocalized with tyrosine hydroxylase labeling for dopaminergic terminals, which either lacked detectable membrane specializations, or formed thin, symmetric synapses in single sections. This suggests that many presynaptic D2 receptors in the striatum represent autoreceptors.

2.1.5. Subcellular distribution of dopamine receptor labeling in the postsynaptic cell

What is the distribution of postsynaptic dopamine receptors in relation to dopaminergic or glutamatergic terminals? Yung et al. (1995) observed D1 and D2 receptor immunoreactivity in membranes of dendrites and spines postsynaptic to terminals forming symmetrical synapses (presumably dopaminergic terminals) and less commonly, asymmetrical synapses. In addition to immunoreactivity associated with synapses, a high proportion of the immunoreactivity was also on membranes at nonsynaptic sites. In agreement with this, a number of immunohistochemical studies of the D1 receptor distribution show that receptor sites are unevenly distributed along the postsynaptic membrane and clusters of receptors are not necessarily postsynaptic to any afferent terminals (Levey et al., 1993; Hersch et al., 1995; Caille et al., 1996). In double labeling experiments using TH and D1 receptor antibodies, Caille et al. (1996) showed that a large majority of D1 positive elements are not apposed to TH-labeled profiles; and when they are, the D1 label is not necessarily concentrated at the portion of the membrane face opposite to TH-profiles.

Consistent with an extrasynaptic location of dopamine receptors, Huang et al. (1992) found D1 receptor labeling in the heads and the necks of spines, as well as in dendritic shafts at postsynaptic sites apposed to symmetric synapses. Similarly, Levey et al. (1993) found both D1 and D2 receptors localized in spiny dendrites and spine heads. Hersch et al. (1995) used subtype specific polyclonal and monoclonal antibodies to label D1 and D2 subtype receptors. Most prominently labeled were spiny dendrites with intense patches of submembranous label sometimes associated with symmetrical or asymmetrical

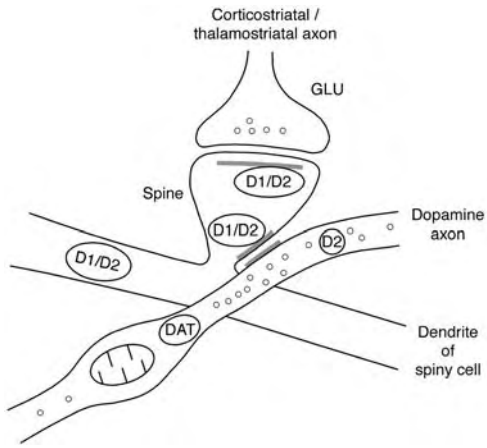

Fig. 3. Localization of dopamine receptors and transporters in relation to pre- and postsynaptic structures. Dopamine transporter (DAT) and dopamine D2 receptors are localized to dopamine axons, but may be some distance from the sites of synaptic contact. Postsynaptic dopamine D1 and D2 receptors (D1/D2) are localized to postsynaptic densities of symmetrical and asymmetrical synapses, and also dendrites. See text for details.

synapses. Within the spines there was diffuse cytoplasmic labeling and also an intense labeling of postsynaptic densities.

In summary, in the evidence presented there is agreement that dopamine receptors are not concentrated immediately within the dopaminergic synapse but are located some distance away, sometimes in association with other types of synapses. Figure 3 summarizes these arrangements of dopamine receptors in relation to pre- and postsynaptic structures.

2.2. SPATIOTEMPORAL DISTRIBUTION OF DOPAMINE

The spatiotemporal distribution of dopamine, after its release by efflux from a synaptic terminal, depends on diffusion and reuptake via the dopamine transporter. Over the past decade, there has been a growing evidence for free diffusion of dopamine from the synaptic cleft and into the surrounding extracellular tissue, a form of synaptic signaling that in other systems has been called volume transmission (Agnati et al., 1995). Dopamine uptake, release and diffusion have been the subject of several recent reviews (Garris and Wightman, 1995; Gonon et al., 2000), and the following represents an emerging consensus of views.

The dopamine transporter is responsible for terminating the dopamine signal. As illustrated in Fig. 3, in the striatum, dopamine transporter labeling is localized both near to and distant from the synaptic specializations, but rarely over the actual sites of the synaptic contact (Nirenberg et al., 1996; Hersch et al., 1997). This predominantly extrasynaptic localization of the dopamine transporter implies that the released dopamine is free to escape from the synaptic cleft into the surrounding extracellular fluid. Thus, the spatial distribution of dopamine in the initial tens of milliseconds after release is mainly determined by diffusion. This is shown by electrochemical studies in which reuptake inhibitors produce only moderate increases in the peak concentration measured in the extracellular space, although the duration of increased concentration is prolonged (Garris et al., 1994; Gonon, 1997). Also, when stimuli are repeated rapidly (e.g. four stimuli in 30 ms)

there is a linear relationship between the number of stimuli and the concentration increase in the extracellular space, which implies that binding to reuptake sites in the synapse does not restrict dopamine efflux (Garris et al., 1994). Thus, most of the released dopamine escapes from the synaptic cleft and into the extracellular space.

Quantitatively, the region of influence of dopamine in the extracellular space around each release site is determined by the interaction of diffusion and reuptake. Mathematical models to describe the interaction between diffusion and reuptake have been developed based on the diffusion theory and the Michaelis–Menten kinetics (Nicholson, 1995). The key factors determining the quantitative spatiotemporal distribution after a single synaptic release include dopamine transporter activity as measured by the Michaelis–Menten parameters *Kd* and *V*max, the diffusion coefficient of dopamine, and the tortuosity of the extracellular space (Nicholson and Tao, 1993). Taking these factors into consideration, Fig. 4 shows the predicted spatiotemporal distribution of dopamine after a release from a single release site. As shown in Fig. 4A, after a single release event, the concentration changes occurring at a receptor a distance of 1.2 μm away are mainly dominated by diffusion. At this distance, the concentration peaks within milliseconds. At greater distances the concentration changes are slower, and a lower peak concentration is released as the transporter activity begins to take effect (Fig. 4B). When release from multiple sites is considered, the half-life of the released dopamine in the extracellular fluid has been

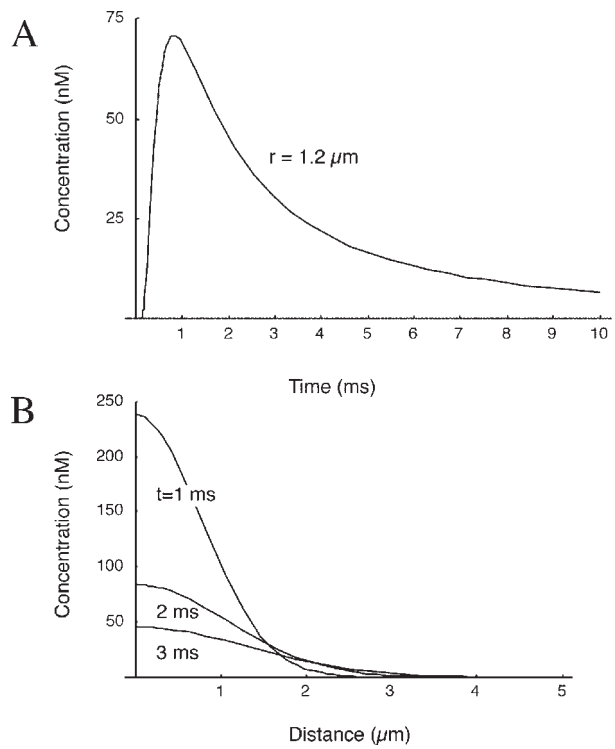

Fig. 4. Calculated dopamine concentration distribution after unitary synaptic release. (A) Dopamine concentration as a function of time at a distance $r = 1.2\,\mu m$ from the release site. (B) Dopamine concentration as a function of distance at time $t = 1$, 2 or 3 ms after release. Based on diffusion coupled with reuptake (Sun, 2002).

estimated to be in the range of 20–30 ms in the striatum (Kawagoe et al., 1992; Gonon et al., 2000). The corresponding diffusion distance at which at least 50% of the released dopamine can diffuse is about 7–10 μm (Wightman and Zimmerman, 1990; Gonon et al., 2000).

How are the individual microspheres of influence of each dopaminergic release site related to one another? This depends on the degree of divergence of the individual axons, and the volume in which the terminals are distributed, from which the nearest-neighbor distances between synapses of the same presynaptic neuron can be estimated.

Dopamine cells in the pars compacta number about 7200 (Oorschot, 1996) whereas we estimate 2.7×10^9 dopamine synapses in the striatum, suggesting that on an average, each dopamine cell must contribute about 370,000 synapses (Table 1). This is a high degree of divergence of individual axons. Failures are thought to be uncommon based on measures of the dopamine efflux after macroscopic stimulation (Garris et al., 1994) but this has not been directly measured for unitary synaptic events.

Given this divergence, what is the influence on the spatiotemporal distribution of dopamine when a single dopamine cell fires an action potential? Unfortunately, the volume of the striatum in which the axons of a single dopamine cell ramify is not known. A qualitative description (Prensa and Parent, 2001) indicates a heterogeneous, but in general a widely distributed axonal arborization. For the sake of argument, we assume that on an average, each dopamine cell innervates a volume in the order of 1 mm^3 (Prensa and Parent, 2001). The average nearest neighbor distance between the 370,000 synapses of a given cell ramifying in this volume would be in the order of 7.7 μm. This figure is larger than the nearest neighbor distance between the dopamine terminals of all the cells estimated above (1.2 μm), because the terminals of a given cell are only a small fraction of the terminals in a volume.

The distance between dopamine synapses of a given cell is remarkably similar to the 7 μm diffusion distance at which at least 50% of the released dopamine can diffuse (Gonon et al., 2000). Thus, individual action potentials are likely to produce gradients of dopamine concentration, varying as a function of distance from release sites. However, the difference in concentration within the field of influence of a single dopamine cell may be as little as two-fold.

2.3. DOPAMINE NEURONE FIRING PATTERNS AND DOPAMINE RELEASE

Electrophysiological recordings from the dopamine cells that were identified antidromically showed a range of firing rates in anaesthetized animals. From the first identified cells the range included silent neurones, slowly firing neurones and neurones firing in short bursts (Deniau et al., 1978; Grace and Bunney, 1984a,b; Dai and Tepper, 1998). A clock-like rhythmic firing mode was also observed in recordings from slices of the mesencephalon (Grace and Onn, 1989). This was initially considered rare in the in vivo, recordings but was a source of fascination nevertheless. The intrinsic cell mechanisms for the generation of this firing mode have been worked out (Lacey et al., 1989; Wilson and Callaway, 2000; Grillner and Mercuri, 2002). Clock-like firing patterns have been observed in anaesthetized (Paladini and Tepper, 1999) and conscious rats (Hyland et al., 2002). They are present in a significant proportion of cells, but in the majority of cells the regular firing pattern is masked, presumably by synaptic inputs to the cells (Hyland et al., 2002). Conversely, the burst-firing mode, common in anaesthesia (Grace and Bunney,

1984b) was not seen in vitro, except after some rather drastic manipulations of the external environment of the slices (Johnson et al., 1992).

From these observations, it is clear that dopamine cells are not silent 'at rest' but that there is an intrinsic tone in the system. This tone may be the basic cellular mechanism underlying the resting release of dopamine, which is responsible for the background level of dopamine detected by dialysis of the extracellular fluid. Such a resting firing rate also implies that the silence of the dopamine cells is an active process, involving inhibitory synaptic process (Paladini and Tepper, 1999). Thus, the effect of a pause in dopamine cell firing on the extracellular concentration of dopamine is important to determine.

So far the discussion of firing patterns of the cells have been restricted to those seen in the most common preparations; but the function of dopamine is surely best studied in conscious animals able to move and respond to external cues. In the past decade such recordings have begun to paint a very intriguing picture of the role of dopamine cells in animal behavior. Early studies of this type had been disappointing from the point of view of the involvement of dopamine cells in motor behavior. In cats (Trulson and Jacobs, 1979; Trulson, 1985) and monkeys (Schultz, 1986) it seemed that the dopamine cells were not responsive to the present behavior of the animal. Few, if any, the cells responded either to the movements in a motor task or to the sensory cues guiding the behavior.

More recent studies in monkeys by Schultz and colleagues have totally reversed this view (Ljungberg et al., 1991, 1992; Mirenowicz and Schultz, 1994, 1996). When monkeys are not first overtrained in the task, then the dopamine cells recorded in the ventral mesencephalon respond to various aspects of the task. Exactly when the dopamine cells fire a burst of action potentials depends on the state of training in the task (Ljungberg et al., 1992). While the monkeys are naïve to the situation the cells respond about 200 ms after the delivery of a reward (Mirenowicz and Schultz, 1994). As the behavior is acquired, this burst of dopamine release is timed to follow stimuli that have come to predict the arrival of the reward, even if the trigger is a movement of the animal itself and not an explicit external cue. As the task is learned, the dopamine burst moves to earlier and earlier predictors of reward (Schultz et al., 1993). Importantly, if a reward is omitted in some trials, the dopamine cells are silenced at the point where an expected reward is not delivered (Mirenowicz and Schultz, 1996). These results appear remarkably consistent with the modern learning theory concepts of reward (Schultz et al., 1993, 1997; Schultz, 1997, 2000; Waelti et al., 2001).

Similar studies in rats have reached *similar* conclusions. Although the exact pattern of responding in individual cells is less homogeneous in the rats, similar general rules apply. The responses in the cells are predictive of future rewards and the absence of a predicted reward leads to a period of silence when it would have been expected (Hyland et al., 2002). In view of the intrinsic membrane properties of the dopamine cells in vitro, the silences have to be thought of as significant inhibitory actions on the cells. It is also important to note that it seems as if all dopamine cells participate to some extent in this activity. Schultz has been unable to distinguish the responses of more medial dopamine cells from those recorded from more lateral placements and indeed usually summarizes his data by adding all the cells recorded in a particular behavioral paradigm together to illustrate the involvement of the cells in the task (Ljungberg et al., 1992; Schultz et al., 1993; Mirenowicz and Schultz, 1994, 1996).

The evidence of dopaminergic activity in the human striatum associated with the subjective effects of cocaine (Volkow et al., 1997a,b, 1999) and by the active participation in computer games (Koepp et al., 1998) goes some way towards suggesting

that the same – or at least similar – properties might be expected from human dopamine neurones.

From these studies we can perhaps make some conclusions that are relevant to the postsynaptic actions, which we want to analyze in this chapter. The resting state of the system is some, probably asynchronous, activity among the population of dopamine cells. The dopamine cells seem to act together to signal the occurrence of an important event that is itself rewarding, or has become so, by association with a reward in a particular situation. This burst of activity usually follows the rewarding stimulus at a predictable time. In cases when the predicted reward does not arrive at the expected time it is followed instead by a cessation of activity in the dopamine cells; by a short reduction of the release of dopamine.

What is the likely effect on target structures of the different modes of firing of dopamine cells outlined above? To address this question it is necessary to estimate the spatiotemporal distribution of the dopamine concentration increases produced by different firing patterns, and combine this with data on the affinities and potencies of target receptors.

As noted, the divergence of the dopamine projection and the extracellular diffusion of dopamine is compatible with the idea that dopamine acts diffusely in space. The location of dopamine receptors and dopamine transporters at sites beyond the synaptic cleft reinforces this idea. However, the action of dopamine is not diffuse in time. Dopamine diffusion and reuptake occur rapidly, so that firing of a single dopamine cell produces a brief, pulsatile increase of dopamine concentration. In the neighborhood of each synaptic contact this dopamine pulse is likely to have a half-life on the order of tens of milliseconds, and a sphere of influence on the order of microns. Due to the close packing of the dopamine release sites, firing of individual dopamine neurons is likely to produce a more-or-less homogeneous increase in the dopamine concentration within the striatal regions of high dopamine innervation.

In this context, in the resting state asynchronous activity among a population of dopamine cells will produce a steady-state background level of dopamine by summation of individual release events. An approximate idea of the fraction of dopamine terminals in a volume that belong to a particular individual dopamine cell is given by the ratio of the density of the terminals of one dopamine cell (estimated to be about 370,000 mm^{-3}), to the density of terminals of all dopamine cells (estimated to be about 104,000,000) (see Table 1). This gives a ratio of about 250:1. Thus, we can imagine that up to 250 different dopamine cells may overlap and thus contribute to the extracellular dopamine concentration in regions of high dopamine innervation. If each of these is firing asynchronously at the background firing rate of 4 Hz, and dopamine has a 25 ms half-life in the extracellular space, the concentration of dopamine at any given point is likely to be an almost constant sum of 25–50 individual release events, with slight fluctuations about an average resting level.

On the other hand, as noted above, dopamine cells seem to act together to signal the occurrence of an important event that is itself rewarding, or has become so by association with a reward in a particular situation. Burst firing of a subpopulation of dopamine cells, especially if firing is time-locked within a few tens of milliseconds, will produce pronounced spatial and temporal summation of dopamine concentration in the extracellular space. In monkeys, such time-locked firing is implied by the population response averaged over many different dopamine cells (Ljungberg et al., 1992). The firing frequency within a burst is sufficiently high to produce summation. In rats, the range of intraburst frequencies in an animal engaged in reward-related tasks is over 30 Hz, on average, and may be as high as 100 Hz on occasion (Hyland et al., 2002).

What is the actual concentration of dopamine at the dopamine receptors associated with each mode of firing of the dopamine cells? The tonic level of dopamine measured using dialysis is 6.5 nM (Sam and Justice, 1996), consistent with predictions that the spatially averaged concentration of extracellular dopamine in the striatum is in the low nanomolar range (Kawagoe et al., 1992).

Measurement of the concentration of dopamine produced by burst firing of the dopamine cells requires high temporal resolution, as transient events are lost if signals are integrated over long time periods. At present, there is a trade-off between the precise chemical identification of the signal and the temporal resolution of the measurement. The time required to collect dialysate samples for chromatographic analysis limits the temporal resolution of microdialysis to seconds or minutes. However, chromatographic analysis provides the best chemical specificity. On the other hand, voltammetry, chronoamperometry and other electrochemical techniques can provide temporal resolution down to the millisecond range, but additional experiments are required to show that dopamine is the main component of the measured signal. Rapid fluctuations in dialysate dopamine levels have been measured with a voltammetric probe in the outlet line of a microdialysis probe. In general, for low temporal resolution situations, results from the two methods are very similar (Lu et al., 1998), suggesting that in the striatum the extracellular voltammetric signals correspond well to the release of dopamine.

Using electrical stimuli to activate a majority of dopamine cells, measurements show that the concentration of dopamine for a single stimulus pulse is 250 nM on an average (Garris and Wightman, 1995). Stimuli which mimic burst firing activity of dopamine cells give rise to higher concentrations due to accumulation of the released dopamine as a result of saturation of dopamine uptake (Chergui et al., 1994).

In order to link the electrical stimulation of dopamine cells more closely with the reward processes, intracranial self-stimulation (ICSS) has been used as a model for reward-related learning. The measurement of increases in extracellular dopamine using ICSS has proven to be difficult. Using voltammetry, Kruk et al. (1998) did not detect significant increases in extracellular dopamine during ICSS. Similarly, using microdialysis, dopamine is not usually detected unless reuptake inhibitors are used (Nakahara et al., 1992). This suggests that in the presence of reuptake, dopamine must be detected rapidly, before being cleared from the extracellular space by reuptake (Young and Michael, 1993). Garris et al. (1997) used fast-scan cyclic voltammetry to measure electrically-evoked extracellular dopamine concentration in freely-moving rats. Stimulation of ICSS sites with 0.4 s trains of biphasic, constant-current pulses (2 ms each phase) produced an increase in extracellular dopamine concentration, which was linearly frequency-dependent. In the nucleus accumbens, concentrations on the order of 400 nM were measured with 50–60 Hz stimulus trains. In subsequent experiments (Garris et al., 1999), rats which did not show dopamine increases after ICSS-like stimulation failed to learn ICSS. In rats in which increases in extracellular dopamine were evoked by stimulation, ICSS was acquired. In these ICSS-responders, single operator-delivered trains produced increases on the order of 100 nM. In untrained animals levels of 1.8 μM were measured during experimenter-delivered trains which were equivalent to trains self-administered during ICSS, but were rarely observed during the ICSS itself. Similar results were obtained in the dorsal striatum (Kilpatrick et al., 2000). Thus, ICSS-like stimuli produce an increase in dopamine concentration during initial learning.

What is the effect of natural reward on dopamine concentration in the striatum? Efforts to measure the phasic increase in dopamine in response to natural rewards and signals

predicting rewards have been reviewed by Wightman and Robinson (2002). A phasic increase in the dopamine signal has been measured in response to a new environment (Rebec et al., 1997a,b). The changes that occurred on entry into new environment lasted only for a few seconds, and were only evident on an initial exposure. A new environment includes a mixture of positive and negative significance for an animal. Food consumption is a purely hedonistic stimulus. There has been extensive debate about changes in extracellular dopamine concentration during food consumption. Richardson and Gratton (1996) found initial increases in dopamine concentration coinciding with consumption of a milk reward, when it was the first earned reward of the session. In the early stages of learning, there was a transient increase at the onset of the light signaling access to a lever providing condensed milk. However, on later days of testing increases were observed as early as 5 min before the start of the light cue. The resolution of the measuring system may not have been sufficient to pick up a brief, phasic increase at the time of the reward signal. Sexual activity has also been reported to increase dopamine concentration in the striatum (Robinson et al., 2001, 2002), but in paradigms where the reward is unsignaled, the precise temporal structure of the associated behavioral state cannot be determined.

Although there have been measurements of dopamine concentration in behaving animals, none could provide data with sufficient temporal resolution to determine the precise timing of dopamine release during natural learning: see review by Wightman and Robinson (2002). Methods with a time resolution on the order of seconds would miss the extremely short pulses of concentration increase predicted by the single-unit recordings from dopamine cells and the time course of release and clearance after a stimulation-evoked release of dopamine.

2.4. AFFINITIES AND POTENCIES OF DIFFERENT TYPES OF RECEPTORS

In order to determine the effect on target structures of the different modes of firing of dopamine cells, it is necessary to apply our estimate of the temporospatial distribution of the dopamine concentration produced by different firing patterns to data on the affinities and potencies of target receptors. The foregoing discussion suggests that the tonic level of asynchronous dopamine cell population activity leads to a steady-state dopamine concentration in the low nanomolar range. Burst firing associated with motivationally significant events leads to a pulsatile increase in dopamine concentration which is more-or-less spatially homogeneous within regions of dense innervation, with limited spatial concentration gradients in less densely innervated regions. Dopamine receptors of both D1-like and D2-like subtypes are found some distance from release sites, suggesting that the dopamine signal for both subtypes of receptors is produced by diffusion from nearby sites; thus, differential activation of D1-like and D2-like receptors by synaptic vs. extrasynaptic dopamine seems unlikely.

In the following section we consider the localization of receptors in relation to single striatal cells. Are dopamine D1-like and D2-like receptors colocalized? If so, are they differentially activated by tonic and burst firing modes?

2.4.1. Colocalization of dopamine receptor subtypes

As noted above, several different subtypes of dopamine receptors have been characterized by molecular methods, and both D1-like and D2-like dopamine receptors are expressed by

striatal neurons. There has been a controversy regarding the degree to which different subtypes of dopamine receptor colocalize on striatal neurons. Some evidence indicates a high degree of segregation, such that striatal neurons can be subdivided into two roughly equal-sized populations, one expressing D1-like and the other expressing D2-like receptors (Gerfen et al., 1995). Another evidence has suggested a significant degree of overlap, with a high proportion of cells expressing both types of receptors (Surmeier et al., 1996).

Initial suggestions of segregation were based on selective labeling of spiny cells according to immunoreactivity for different receptor subtypes. According to Levey et al. (1993), only a subset of spiny dendrites and neurons displayed immunoreactivity for either receptors. Several different studies support this. Labeling for the dopamine D1 receptor was localized in 49% of striatal neurons and in spiny dendrites (Huang et al., 1992). Hersch et al. (1995) used subtype specific polyclonal and monoclonal antibodies to label the D1 and D2 subtype receptors. By electron microscopy, 53% of the striatal projection neurons were labeled by the D1 subtype receptor antibodies. In a separate section, 48% of the neurons were labeled by the D2 subtype receptor antibodies. When both the receptor antibodies were applied to the same striatal section, about 78% of the cells were labeled. These results suggests that 23% of the striatal projection neurons colocalized both dopamine receptor subtypes.

A higher degree of colocalization is suggested by the most recent studies using the single-cell RT-PCR techniques. Surmeier et al. (1996) found that although colocalization of the D1 and D2 subtypes of dopamine receptors was limited, functional D1 and D2 class receptors were colocalized in nearly 50% of spiny projection neurons. This figure is somewhat less than earlier estimates of colocalization based on single-cell RT-PCR (Surmeier et al., 1992b) which dramatically overestimated the degree of colocalization. The more recent results show high degree of colocalization of the D1 and D2 receptors in neurons which also colocalized substance-P and enkephalin, which are markers of striatonigral and striopallidal neurons (Surmeier et al., 1996).

An even higher degree of colocalization has been reported in studies using confocal microscopy to detect immunofluorescence for D1 and D2 receptors. These studies have found both types of receptors in virtually all striatal neurons. In cell cultures, D1 and D2 receptors have been colocalized to terminals of intrinsic neurons (Wong et al., 1999). Similarly, Aizman et al. (2000) found that cultured striatal neurons expressed both D1 and D2 receptors. These results are not peculiar to the culture situation. When examined in acutely prepared slices, virtually all cells were positive for both the D1 and D2 subclasses.

Aizman et al. (2000) suggested the high degree of apparent colocalization could be reconciled with earlier findings showing segregation, by assuming that the striatonigral neurons contain high levels of D1 and low levels of D2, and the converse is true for the striopallidal pathway. The low proportion of cells exhibiting colocalization of the two subclasses when immunocytochemistry or in situ hybridization is used may be attributable to relatively lower sensitivity of these methods when compared to confocal methods with extremely high sensitivity. If this is the case, then it is also important to ask how the sensitivity of these detection methods relates to the biologically relevant levels of expression. Does the biological system respond whenever there is *any* detectable level of message? Are the naturally occurring levels of dopamine sufficient to produce both D1-like and D2-like responses in *all* cells with a low level of expression?

At the biochemical level, the actions of dopamine at D1 and D2 receptors are antagonistic, with D1 receptor stimulation stimulating cAMP formation and D2 receptor stimulation inhibiting cAMP formation. If in the same cell, both receptors are expressed

and responsive to dopamine, one might expect these two effects to cancel out. Previously, it has been argued that dopamine D2 receptors have several orders of magnitude, higher affinity for dopamine than the dopamine D1 receptors. The argument then goes that basal levels of dopamine might activate the D2 receptor, and higher concentrations would be required to activate the D1 receptor. The low average concentrations produced by the tonic firing pattern of dopamine cells might thus activate high affinity D2 receptors, while burst firing modes would activate low affinity D1 receptors. However, this argument must also take into account that virtually all G-protein coupled receptors exist in high and low affinity states and this is a completely fluid phenomenon (Leff et al., 1985). What state any particular receptor is in depends, basically, on the total microenvironment.

The existence of both high and low affinity states has been demonstrated for both D1 and D2 receptors (Richfield et al., 1989). The high affinity states for either receptor have similar affinity for dopamine, in the nanomolar range, with D1 being slightly more sensitive. For example, the Ki value for inhibiting the binding of agonists to D1 receptors (30–40 nM) is about one-third that of the D2 receptors (80–120 nM) (Ross, 1991). Seeman et al. (1985) reported much higher affinities, Leff et al. (1985) and Leff and Creese (1985) reported similar values, whereas Flaim et al. (1985, 1986) reported lower affinity. Hamblin et al. (1984) and Hall and Sallemark (1987) reported similar values for D2 receptors. The potency of low nanomolar concentration of dopamine acting on D1 receptors expressed in CHO cells (making cAMP) is compatible with such affinity (Perachon et al., 1999). Thus, it is not possible to make the argument that due to different affinities of dopamine D1 and D2 receptors, differential effects of dopamine on the D1 vs. the D2 receptors, can be brought about by different concentrations. To make such an argument, it is necessary to take into account the proportion of each type of receptor in each affinity state.

Functional colocalization of dopamine receptors is, however, supported by physiological studies that have shown responses mediated by both D1-like and D2-like receptors in a majority of cells tested (Uchimura et al., 1986; Ohno et al., 1987; Hu and Wang, 1988; Surmeier et al., 1992b, 1996; Aizman et al., 2000). In contrast, studies of the molecular effects of dopamine expression have shown segregation of D1- and D2-like responses (Gerfen et al., 1990, 1995, 1998; Keefe and Gerfen, 1995; Berke et al., 1998; Gerfen, 2000). Thus, it is important to ask whether the methods used to elicit these responses reflect normal, physiological, pharmacological or purely experimental conditions.

In the electrophysiological experiments, dopamine and specific dopamine receptor agonists are typically applied in pharmacological concentrations, and often in the presence of reuptake inhibitors. The prolonged and intense stimulation of receptors under these conditions may ensure that even those cells with very few receptors of a particular subtype are able to respond. The effects of endogenous dopamine, released in naturally occurring pulses at a lower concentration may be more selective. Thus, colocalization of receptors as observed by highly sensitive methods is reflected in the responses to exogenous dopamine agonist application, whereas the effects of naturally released dopamine may reflect the segregation of receptors as observed with less sensitive methods.

3. PHYSIOLOGICAL EFFECTS OF DOPAMINE

The effects of dopamine on striatal neurons can be considered along two time scales, a dynamic one and a persistent one. The dynamic time scale refers to relatively immediate

onset and rapidly reversible effects of dopamine. These immediate and reversible effects involve a complex modulation of the voltage-gated and receptor-operated ion channels, which occur during dopamine receptor stimulation. These effects do not appreciably outlast the presence of the agonist. They thus require the continued presence of dopamine for the expression of responses. The persistent, longer-term effects involve dopamine-dependent functional and structural plasticity of the corticostriatal synapses. These effects appear to require dopamine for their induction but not necessarily for their expression, and may be especially relevant to pulsatile changes in dopamine concentration.

At present, it is not known how the dynamic effects and the long-lasting effects combine into a coordinated, integrative action of dopamine. Presumably there is some underlying synergy which produces a meaningful overall response, but its exact nature remains unclear. We propose, firstly, that the immediate and reversible effects are related to the neural mechanisms underlying the behavioral response to incentive stimuli, and the persistent effects are related to the neural mechanisms underlying reinforcement in reward-related learning. Secondly, we suggest that the persistent effects may be selectively amplified by the dynamic effects, and conversely, the dynamic effects may facilitate induction of the persistent effects, with both sets of responses combining to give an overall integrated response.

Extracellular recordings have shown a mixture of effects in response to iontophoretically-applied dopamine. Although some cells showed excitatory responses, over all studies indicate that the great majority of cells showed a decrease in spontaneous or glutamate-induced firing (Bloom et al., 1965; McLennan and York, 1967; York, 1967).

Intracellular recordings have also revealed a similar mixture of effects in response to dopamine, with a bias towards inhibition at high concentrations. In one of the earliest intracellular studies of the effects of dopamine on striatal cells, Kitai et al. (1976) found that dopamine, iontophoretically ejected into the extracellular fluid, produced a brief depolarization. This response to brief iontophoretic currents was similar to an excitatory postsynaptic potential (EPSP), with a time course in tens of milliseconds. However, Herrling and Hull (1980) were unable to replicate these results. Instead they found the effect of iontophoretically-applied dopamine differed according to the distance between the opening of the dopamine ejecting pipette and the tip of the recording electrode. When the tip of the ejecting pipette was located 100 μm away from the tip of the recording electrode, the effect of dopamine was a slow depolarization, which started 5–15 s after the onset of the iontophoretic ejection current. When the intertip distance was 50 μm, the majority of the cells continued to display the slow depolarization, but 30% responded to dopamine application with a hyperpolarization. Herrling and Hull (1980) found they were unable to reproduce any effects with the iontophoretic current pulses used by the earlier investigators. They noted that direct current effects (artefacts of the iontophoretic ejection method) would be more likely to occur with the intertip distances employed in the earlier study (20–40 μm).

An excitatory effect of dopamine has also been reported by Akaike et al. (1987), using striatal slices, in response to bath application of dopamine. They found that dopamine (1 μM) produced a depolarization of about 10 mV amplitude, and lowered the current required to elicit action-potential firing. These effects were blocked by the D2 antagonist domperidone, a selective D2 antagonist. Higher concentrations of dopamine (10–100 μM) raised the threshold for action potential generation. These high concentration effects were blocked by the D1 antagonist SCH 23390.

The inhibitory effect of dopamine on firing evoked by current injection is supported by studies by Calabresi et al. (1987a). These showed that dopamine reduced the number of action potentials evoked by the depolarizing intracellular current injection. This effect was due to a reduction of a tetrodotoxin-sensitive inward current (presumed Na^+ current), and was evident as a reduction of the gradual ramp potential before each spike. This effect was mediated by dopamine D1 receptors (could be blocked by SCH 23390, mimicked by SKF 38393). Dopamine also reduced the amplitude of the intrastriatally evoked postsynaptic potential, but only at depolarized potentials, not at hyperpolarized potentials. This effect of dopamine was thought to be due to reduction of the same persistent Na^+ conductance as described above.

Similarly, Rutherford et al. (1988) found that iontophoretically-applied dopamine reduced the number of action potentials evoked by depolarizing current pulses. In addition they found that dopamine inhibited the afterhyperpolarization that followed the trains of action potentials.

3.1. DOPAMINE MODULATION OF ION CHANNELS

The immediate and reversible actions of dopamine are a combination of modulations of individual ion channels. The major ion channels expressed in spiny projection neurons are summarized in Table 2. Current understanding of the properties of these channels is mostly based on whole cell recordings from isolated cells, which have been recently reviewed by Nichola et al. (2000). The role of these channels in whole cell behavior has been studied using intracellular recordings in brain slices or anaesthetized animals. Many of the important cellular properties of spiny projection neurons can be accounted for in terms of ion channel activations occurring at different membrane potentials.

TABLE 2. *Electrophysiologically characterized currents in spiny projection neurons*

Electrophysiologically-defined current	References
I_{Kir} (Inward rectifier type K^+ channel)	Hagiwara and Takahashi (1974); Leech and Stanfield (1981); Uchimura et al. (1989); Nisenbaum and Wilson (1995a)
I_{As} (Slowly inactivating A-type K^+ channel)	Surmeier et al. (1991, 1992b); Nisenbaum et al. (1994); Nisen baum and Wilson (1995a); Gabel and Nisenbaum (1998)
I_{Na} (Na^+ channel)	Ogata and Tatebayashi (1990); Surmeier et al. (1992a); Fraser et al. (1993); Hoehn et al. (1993); Cepeda et al. (1995); Chao and Alzheimer (1995); Schiffmann et al. (1995)
L (Noninactivating, high voltage activated Ca^{++} channel)	Bargas et al. (1991, 1994)
N, P (Inactivating, high voltage activated Ca^{++} channel)	Bargas et al. (1994); Surmeier et al. (1995)
I_{Krp} (Resistant, persistent A-type K^+ channel)	Nisenbaum et al. (1996)
I_{Af} (Rapidly inactivating A-type K^+ channel)	Surmeier et al. (1988, 1989)

It should be acknowledged that understanding of the effects of dopamine on whole cell behavior, in terms of modulatory effects on ion channels, is at a more preliminary and somewhat speculative stage. Data on the modulation of individual channels by dopamine now has to be put in the perspective of the membrane potential fluctuations of the whole cell. A detailed and quantitative analysis is crucial to understanding the modulatory actions of dopamine on membrane currents, because exactly which currents are available depends on the recent history of the cell, for this is what determines which of the many currents are turned on and thus available for modulation by dopamine.

The typical firing activity of the striatal spiny neurons in awake animals consists of brief episodes of firing separated by longer periods of relative inactivity (Schultz and Romo, 1988; Kimura et al., 1990). Such episodes of firing are associated with initiation, execution, or termination of particular movements on the part of the animal (Alexander, 1987; Schultz and Romo, 1988; Kimura et al., 1990). These firing patterns of striatal spiny neurons also occur in immobilized, locally anaesthetized rats (Wilson and Groves, 1981) and in the urethane-anaesthetized rats (Wilson, 1993). Large amplitude membrane potential fluctuations from a hyperpolarized Down state to a depolarized Up state appear to be necessary for action potential firing in striatal neurons (Wilson and Groves, 1981; Wilson and Kawaguchi, 1996). These Up state transitions are not intrinsic oscillatory behaviors of the spiny cells but require synaptic input from the cerebral cortex and the thalamus. Up state transitions do not occur after removal or deactivation of the cortex (Wilson et al., 1983) or in brain slices in which coordinated cortical activity has been interrupted (Arbuthnott et al., 1985, Kawaguchi et al., 1989). On the other hand, Up state transitions do occur spontaneously in the cortex-striatum cocultures, in which there is an intrinsic activity of the cortical explant (Plenz and Aertsen, 1996; Kerr and Plenz, 2002). Similarly, cortical stimulation in the intact animal can evoke depolarizing events very similar to the Up state transitions that occur spontaneously (Wilson, 1995b; Wilson and Kawaguchi, 1996). Thus, corticostriatal inputs are both necessary and sufficient for Up state transitions.

In brain slices in which spontaneous cortical activity does not occur, spiny projection neurons remain at a stable, relatively hyperpolarized Down state resting membrane potential close to the K^+ equilibrium potential. This hyperpolarized potential is largely due to a powerful, inwardly rectifying K^+ channel, I_{Kir} (Calabresi et al., 1987b; Uchimura et al., 1989). This voltage-sensitive potassium conductance is active at the resting membrane potential and becomes inactivated as the membrane is depolarized, similar to the current described in starfish (Hagiwara and Takahashi, 1974). In spiny cells it accounts for the low input resistance and short membrane time constant at hyperpolarized membrane potentials, which act to shunt excitatory inputs, thereby maintaining the membrane potential in the hyperpolarized state. In contrast, when a spiny cell receives coordinated inputs, such as those that occur during a barrage of cortical afferent input (Stern et al., 1997, 1998), I_{Kir} will begin to deactivate. As deactivation occurs the input resistance and time constant of the cell increase, permitting greater temporal and spatial summation of excitatory inputs (Nisenbaum and Wilson, 1995a,b). Clearly, this current plays a major role in the subthreshold behavior of the cell.

As noted in Table 3, the current responsible for inward rectification, I_{Kir} (Mermelstein et al., 1998), is increased by D1 receptor activation (Galarraga et al., 1994; Pacheco-Cano et al., 1996). In contrast, D2 receptors suppress I_{Kir} currents (Uchimura and North, 1990) although they also activate a low conductance channel (Freedman and Weight, 1988, 1989; Greif et al., 1995; Lin et al., 1998; Waszczak et al., 1998). Thus, dopamine acting via D1

TABLE 3. *Dopamine receptor subtype-specific effects*

Channel	Dopamine D1 receptor activation	Dopamine D2 receptor activation
I_{Kir}	Increased (Galarraga et al., 1994; Pacheco–Cano et al., 1996)	Increased (Freedman and Weight, 1988, 1989) or decreased (Uchimura and North, 1990)
I_{As}	Decreased (Surmeier and Kitai, 1997)	Increased (Surmeier and Kitai, 1997)
I_{Na}	Reduced (Surmeier et al., 1992a)	Reduced by D3; increased by D2 (Surmeier et al., 1992a)
L	Increased (Surmeier et al., 1995; Hernandez–Lopez et al., 1997)	Decreased (Hernandez–Lopez et al., 2000)
N, P	Decreased (Surmeier et al., 1995)	Decreased (Surmeier et al., 1995)

receptors increases I_{Kir} and holds the cell in the Down state; via D2 receptors dopamine is likely to decrease I_{Kir} and release the cell from the Down state.

In response to the near-threshold constant current, the membrane potential of spiny projection neurons exhibits a gradual ramp-like depolarizing trajectory and a long-latency to spike discharge, after which relatively regular action potential firing occurs. During the ramp-like depolarization, the slowly inactivating A-type K^+ channel I_{As} (Nisenbaum et al., 1994) competes with the inward Na^+ and Ca^{++} currents, and acts to slow the rate of depolarization, giving rise to the ramp potential and delayed spike discharge (Nisenbaum and Wilson, 1995b; Wilson, 1995a). The availability of this I_{As} current to influence the membrane potential fluctuations seen in vivo, depends dramatically on the recent history of the cell. Thus, if the cell has been in the hyperpolarized state for a long period before receiving an excitatory synaptic barrage, then much of the inactivation of I_{As} will have been removed, so that it is available to reduce the level of the response. In contrast, if the cell has been in the hyperpolarized state for a brief period of time, the I_{As} will be mostly inactivated, permitting a larger response to the synaptic input (Nisenbaum et al., 1994).

Dopamine and the specific D1 agonist SKF 38393 (5 μM) reduce I_{As} (Kitai and Surmeier, 1993; Surmeier and Kitai, 1993). Conversely, the D2 agonist quinpirole (5 μM) enhances I_{As} (Surmeier and Kitai, 1997). Due to the voltage-dependent activation and inactivation of I_{As}, these D1-mediated effects of dopamine should depend upon the membrane potential range in which the neuron is operating. If in the Down state, or early in the Up state, then a considerable fraction of I_{As} will be available. In this state, dopamine acting through D1 receptors should decrease the strength of this current. This should facilitate depolarization in response to cortical inputs. Thus, dopamine acting via D1 receptors enables a transition from the Down state to the Up state.

The effects of dopamine on the potassium channels discussed appear to oppose each other, in that I_{Kir} is increased while I_{As} is decreased. The former effect is to stabilize the Down state, whereas the latter effect is to facilitate the transition to the Up state. The combination of these effects may be to make the spiny neurons reluctant to change states, but more snappy about doing so if their inputs are increased or decreased by a large enough amount.

Slow and persistent Na^+ channels represented by I_{Na} are responsible for regenerative events underlying subthreshold ramp depolarizations and action potential firing in spiny projection neurons. This current normally produces a depolarizing prepotential, just before the action potential. The prepotential is sensitive to the sodium-channel blocker, TTX; but not to calcium channel blockers (Bargas et al., 1989). It is responsible for the

later part of the slow rise in membrane potential seen during positive direct current injections (Bargas et al., 1989).

Dopaminergic modulation of Na^+ channels is a probable mechanism for the inhibitory effects reported in intracellular studies. As noted above, the amount of injected current required to reach the threshold voltage for action potential generation is increased by dopamine in a dose-dependent manner (Calabresi et al., 1987a). A dopamine D1-receptor mediated reduction of the depolarizing prepotential by dopamine was proposed as the mechanism underlying this inhibitory effect (Calabresi et al., 1987a, 1988). Voltage-clamp studies in dissociated striatal cells have confirmed that dopamine D1 receptor activation causes a reduction in peak Na^+ current, which may be with or without a shift in voltage dependence of inactivation (Surmeier et al., 1992a; Schiffmann et al., 1995, 1998). The effect of these changes is likely to increase the delay of firing of spiny neurons.

Dopamine acting via the D2 receptors has complex effects on Na^+ currents. An increase in the amplitude of this current has been reported in a minority of cells (Surmeier et al., 1992a). These currents are also reduced in response to a D2 receptor activation by means of a negative shift in voltage dependence of steady-state inactivation (Surmeier et al., 1992a). In cells in which a D2-mediated decrease in Na^+ current was measured, the decrease was due to a shift in the voltage dependent inactivation towards more hyperpolarized potentials. This would make no difference at hyperpolarized Down state potentials but a big difference at more depolarized Up state potentials, where the effect would be to reduce the Na^+ current in most cells.

Spiny neurons express an extensive range of calcium currents, including L-, N-, P-, Q- and R-type Ca^{++} channels (Mermelstein et al., 1999). With maintained depolarization, the depolarization-activated K^+ currents begin to inactivate, and inwardly-rectifying currents shut off. At this stage in the cycle of repetitive firing high-voltage activated Ca^{++} channels begin to activate (Bargas et al., 1991, 1994; Surmeier et al., 1995). The Ca^{++} channels have the effect of increasing the duration of the action potential and facilitating the entry of calcium into the cell. The dendritic entry of calcium is a function of both afferent activity and membrane potential (Kerr and Plenz, 2002). Although depolarization associated with Ca^{++} entry helps to maintain the depolarized state, the high voltage of activation of these channels suggests a primary role in controlling intracellular calcium.

Dopamine effects on Ca^{++} channels are complex. Dopamine D1 receptor activation reduced N- and P/Q-type Ca^{++} currents but enhanced L-type currents (Surmeier et al., 1995). This was apparent in a much greater proportion of cells recorded with sharp electrodes (Hernandez-Lopez et al., 1997) arguing for a dendritic location. D1 receptor activation prolonged Ca^{++} plateau potentials in the presence of the potassium channel blocker, tetra-ethyl ammonium (TEA), an effect which was occluded by the calcium channel agonist BAY K8644, resulting in an increased repetitive firing and prolonged AP duration.

On the other hand D2 receptor stimulation in enkephalin-expressing medium spiny neurons suppresses Ca^{++} currents through L-type Ca^{++} channels (Hernandez-Lopez et al., 2000). Suppression is not mediated by inhibition of adenylate cyclase.

3.1.1. Synthesis of channel effects on whole cell behavior

Although there is not yet sufficient information to achieve a total synthesis of the effects of dopamine on ion channels and striatal cell activity, it seems useful to attempt to put

together what is known in relation to whole cell behavior. The membrane potential trajectory in response to a depolarizing current pulse reflects the activation and inactivation of many of the currents modulated by dopamine. At the onset of a depolarizing current pulse, the membrane begins to depolarize. As it does so, the I_{Kir} is turning off. As the membrane depolarizes further, the fast and slow potassium currents begin to activate. The fast component is not known to be dopamine-sensitive and is not considered here. The slow Na^+ current activates as the membrane potential approaches threshold. At the same time the I_{As} begins to inactivate. As the cell begins to fire, the L channels activate with each action potential.

The dopamine-mediated increase of I_{Kir} increases the stability of the hyperpolarized state of the cell. The decrease of I_{Na} reduced the prepotential and also reduces excitability. These two effects together produce a less excitable cell, in which it is more difficult to effect a transition from the Down state to the Up state. Opposing these effects the decrease in I_{As} and the increase in L channels mean that if the depolarized state is prolonged, D1 activation increases excitability. These conclusions broadly agree with those of Calabresi (1987a), see Fig. 4; and Hernandez-Lopez (1997), see Fig. 1. Under conditions of prolonged depolarization, D1 receptor stimulation may thus lead to increased action potential firing, as observed in vivo, (Gonon, 1997; West and Grace, 2002).

The effects of D2 activation are more speculative at present, but essentially seem to be the reverse of the effects for D1. Decreasing I_{Kir} would be expected to decrease the stability of the Down state. An increase of I_{Na} would increase the excitability of cells in the Up state. This effect may be opposed by an increase in I_{As} and a decrease in L-channels leading to a delay in firing.

3.2. DOPAMINE EFFECTS ON SYNAPTIC TRANSMISSION

In addition to immediate short-term effects on channel properties, dopamine also plays a key modulatory role in the regulation of neuronal responses mediated by activation of excitatory amino acid receptors. The nature of the modulatory effects of dopamine depend on the excitatory amino acid receptor subtype and the specific dopamine receptor subtype activated. The modulation of NMDA and AMPA receptors by dopamine D1 and D2 receptors has been reviewed recently by Cepeda et al. (1998) and Di Chiara et al. (1994).

Modulatory effects may be postsynaptically mediated by interactions within the spiny projection neurons, or involve presynaptic regulation of neurotransmitter release from corticostriatal terminals. Postsynaptic effects may be mediated by direct actions of intracellular signaling pathways (cAMP, calcineurin) on receptor status (phosphorylation/dephosphorylation of receptor proteins), and actions on voltage-dependent channels, which may amplify or attenuate the electrical response of the cell to synaptic currents.

Dopamine D1 receptor activation enhances NMDA-mediated excitatory responses (Cepeda et al., 1993; Cepeda and Levine, 1998). The modulatory actions of dopamine on NMDA receptor mediated responses are reduced in D1 deficient mice (Levine et al., 1996a), supporting a specific role for D1 receptors in enhancement. However, this enhancement involves a complex interplay of actions both on the NMDA receptors and also on the voltage-sensitive calcium channels (VSCC). In particular, the activation of VSCC conductances on the distal dendrites contributes to the enhancement of NMDA currents by dopamine. This mechanism of enhancement involves increased regenerative amplification of synaptic responses by increased VSCC currents (Cepeda et al., 1993, 1998; Cepeda and Levine, 1998). Synaptic responses may also be increased directly by

phosphorylation of NMDA receptor subunits (Flores-Hernandez et al., 2002). Dissecting the relative contribution of the NMDA and the VSCC changes is complicated by space-clamp difficulties when intact cells are studied, as both conductances are expressed on dendrites. On the other hand, it seems clear that changes in K+ conductances do not make a major contribution, as blockade of K+ conductances does not prevent dopaminergic enhancement of NMDA currents (Altemus and Levine, 1996).

Dopamine D1 receptor activation has also been reported to increase AMPA receptor currents in cultured striatal neurons (Price et al., 1999). In brain slices, the effect of dopamine D1 receptor activation on nonNMDA receptor-mediated synaptic responses is variable, with reports of potentiated synaptic responses in a large fraction of cells (Cepeda et al., 1993) or variable effects, but with more increases than decreases reported (Levine et al., 1996b; Levine and Cepeda, 1998).

In contrast to the potentiating effects of dopamine D1 receptor stimulation, D2 receptor activation attenuates responses evoked by both NMDA and nonNMDA receptor agonists (Cepeda et al., 1992, 1993). Conversely, glutamatergic transmission is increased in D2 and D4 receptor knockout mice, compared to the wild-type mice, consistent with an inhibitory effect of D2 like receptors on synaptic transmission (Cepeda et al., 2001).

The net effect of dopamine is thus likely to depend on the degree of activation of D1 and D2 receptors, on the contribution of NMDA and AMPA receptor-operated channels to the synaptic response, and the interplay between VSCCs and synaptic responses. Generally, the D2 effect predominates, i.e. dopamine inhibits depolarization and firing evoked by glutamate (Cepeda et al., 1992, 1993).

3.3. DOPAMINE-DEPENDENT PLASTICITY OF CORTICOSTRIATAL SYNAPSES

At the molecular level, dopamine and glutamate produce cooperative effects on gene expression in a subset of striatal neurons (Berretta et al., 1992), suggesting they may affect neuronal activity over extended periods of time. Long-lasting functional effects of interactions between dopamine and glutamate have also been measured at the electrophysiological level. Extracellular recordings from neostriatal neurons in awake, behaving animals show long-lasting changes in activity patterns related to the acquisition and performance of learnt behavior. New responses to task-related stimuli are acquired during learning (Kawagoe et al., 1998; Shimo and Hikosaka, 2001; Lauwereyns et al., 2002; Takikawa et al., 2002) and such acquired responses persist as long as performance is maintained (Aosaki et al., 1994b). The acquisition of behavioral and neuronal responses is dependent on the nigrostriatal dopamine system (Aosaki et al., 1994a). As noted, the nigrostriatal dopaminergic neurons are activated during the learning of behavioral actions (Schultz et al., 1993) in relation to positive reinforcement (Mirenowicz and Schultz, 1996). Together, these findings suggest that the dopamine afferents are involved in long-lasting changes in neural responses that occur in the neostriatum in association with learning.

Dopamine-dependent synaptic plasticity of the corticostriatal pathway is a probable basis for the long-lasting changes in neuronal responses described in the neostriatum. Synaptic plasticity is a long-lasting change in the functional efficacy of synaptic connections that is induced by certain patterns of brain stimulation. It is widely used as an experimental model for learning and memory mechanisms of the brain (Bliss and Collingridge, 1993). Several authors have proposed that synaptic plasticity mechanisms

underlie learning-related effects of dopamine in the neostriatum (Beninger, 1983; Miller, 1988; Wickens, 1990; Wickens and Kotter, 1995).

Experimental study of synaptic plasticity in the neostriatum has advanced rapidly and dopamine-dependent synaptic plasticity in the striatum has been reviewed recently by Reynolds and Wickens (2002). Both long-term potentiation (LTP) and long-term depression (LTD) of the synaptic responses have been described in the striatum.

Long-term depression can be induced in the synapses connecting the cerebral cortex to the neostriatum by high-frequency stimulation (HFS) of the cerebral cortex (Walsh, 1991; Calabresi et al., 1992a,b,c 1993, 1994; Lovinger et al., 1993; Walsh, 1993; Walsh and Dunia, 1993; Kombian and Malenka, 1994). It is a depolarization-dependent process that requires activation of voltage-sensitive calcium channels in the postsynaptic cell during the conditioning tetanus (Calabresi et al., 1992b, 1994). Activation of glutamate metabotropic receptors is also a requirement, but activation of NMDA receptors is evidently not required.

Of particular relevance to the effects of dopamine on synaptic plasticity, Calabresi et al. (1992a, 1994) found that LTD could not be induced in slices prepared from dopamine-depleted animals, but could be restored by bath application of exogenous dopamine, or coapplication of both D1 and D2 dopamine receptor agonists. An LTD could be prevented from occurring in normal slices by pretreatment with either D1 or D2 antagonists. Thus, coactivation of D1 and D2 dopamine receptors appears to be a requirement for LTD induction. The dopamine level brought about by electrical stimulation of brain slices from normal animals is apparently what provides sufficient stimulation of D1 and D2 receptor to support LTD. Surprisingly, in contrast to the slice results, in vivo LTD is not abolished by depletion of releasable dopamine by alpha-methyl-para-tyrosine (Reynolds and Wickens, 2000) or blockade of dopamine D1 receptors with SCH23390 (Floresco et al., 2001).

Long-term potentiation also has been reported in the striatum. Striatal LTP appears to be a dopamine-dependent form of potentiation. It should be emphasized that HFS trains, which would be expected to induce potentiation in other glutamatergic circuits, were initially found to produce only LTD in the striatum. Initial reports of striatal LTP were based on the effects of HFS in slices bathed in magnesium-free fluid (Calabresi et al., 1992c) and regarded as a pathological phenomenon (Calabresi et al., 1996). Under Mg-free conditions there is greater activation of NMDA receptors, by removal of a voltage-dependent Mg^{++} block. Naturally, it has been suggested that an increased influx of calcium into the postsynaptic neuron under these conditions favours LTP over LTD, as in other systems. Consistent with this, striatal LTP is blocked by NMDA antagonists and by intracellular EGTA (Calabresi et al., 1992c). However, while NMDA receptor activation is required for induction of Mg-free LTP, this does not necessarily mean that LTP is due to increased entry of Ca^{++} through NMDA channels, as in other systems. Instead, there is evidence that Mg-free LTP facilitates LTP by a dopamine-dependent mechanism. Both dopamine depletion and the dopamine D1 receptor antagonist SCH23390 can block LTP in magnesium-free fluid (Kerr and Wickens, 2001).

In the light of our data showing LTP to be dopamine-dependent, it seems likely that the facilitation of LTP in Mg-free conditions may be brought about by increased HFS-induced release of dopamine. Increased dopamine release occurs in Mg^{++}-free solution due to activation of presynaptic NMDA receptors, presumably located on dopaminergic nerve terminals (Roberts and Sharif, 1978; Krebs et al., 1991a,b; Desce et al., 1992). As noted above, the dopaminergic terminals on spiny projection neurons synapse in close

proximity to the glutamatergic corticostriatal terminals (Freund et al., 1984; Smith et al., 1994; Hersch et al., 1995; Yung et al., 1995). Thus, glutamate released from corticostriatal terminals during cortical HFS might act directly on adjacent dopaminergic terminals to cause dopamine release. Spillover of glutamate under Mg^{++}-free conditions favours NMDA-mediated release of endogenous dopamine.

Another facet of striatal LTP is that potentiation is reliably induced by HFS in the presence of the potassium-channel blocker, tetra-ethyl ammonium (Walsh, 1991; Wickens et al., 1998). By analogy with other systems, this result might be interpreted as an increased influx of calcium into the postsynaptic neuron. However, this does not appear to be the case. Intracellular application of potassium-channel blockers does not facilitate LTP (Wickens et al., 1998), as would be predicted, if these effects were mediated by greater depolarization of the postsynaptic neuron. The facilitation of LTP by extracellular potassium-channel blockers is therefore more likely to be due to presynaptic effects, such as facilitation of dopamine release by prolongation of the action potential in dopaminergic axon terminals.

The requirement for dopamine in corticostriatal LTP raises the question of whether dopamine, applied in a manner which mimics the natural release of dopamine produced by reward, is sufficient to facilitate LTP. Using the in vitro intracellular recording techniques, we have demonstrated a significant effect of pulsatile dopamine application on synaptic plasticity in the striatum (Wickens et al., 1996). These experiments employed a conditioning protocol in which presynaptic corticostriatal fibers were stimulated in conjunction with activation of the postsynaptic neostriatal neuron. In the control group this caused depression of synaptic responses. When dopamine was applied in brief pulses coinciding with the pre- and postsynaptic conjunction of activity, the depression was reversed and potentiation of responses was induced (Wickens et al., 1996). Thus, pulsatile application of dopamine reverses the long-term depression which normally follows high-frequency stimulation of the cortex. Further studies have indicated strict temporal requirements for the effects of dopamine on synaptic plasticity (Wickens, 2000).

A number of groups have reported variability in the induction of LTP by HFS, including LTP, LTD and no change with the same HFS protocol (Akopian et al., 2000; Partridge et al., 2000). Variability in the direction and extent of the effect suggests an uncontrolled variable, and efforts have been made to identify the causes of this variability. The location of the postsynaptic neuron (Akopian et al., 2000; Partridge et al., 2000) is a possible factor. It is plausible that location effects are mediated by regional differences in dopamine innervation or dopamine receptor expression (Allin et al., 1989; Russell et al., 1992), and dopamine depletion eliminates mediolateral differences in striatal synaptic plasticity (Smith et al., 2001). There are also regional differences in glutamate release that may contribute to regional differences in synaptic plasticity (Akopian et al., 2000). To resolve such differences, it is important to ensure that corticostriatal stimuli are selective for this pathway and not activating intrastriatal terminals by direct current spread.

The use of intracellular recording in whole-animal preparations has enabled greater separation of stimulating electrodes and more specific activation of afferents than is possible in brain slices. Using this method, HFS of the cerebral cortex induces LTD of the corticostriatal pathway, as in slices. When stimulation of the substantia nigra pars compacta with 20 Hz trains is paired with cortical HFS, a short-lasting potentiation is induced (Reynolds and Wickens, 2000). This short-lasting potentiation is blocked by dopamine depletion. Thus, the phasic activation of dopamine afferents induced

potentiation in vivo, although this was less enduring than the effect of pulsatile application of dopamine seen in vitro (Wickens et al., 1996).

Experiments using extracellular single unit recordings of nucleus accumbens neurons in combination with chronoamperometric measures of dopamine efflux lead to a similar conclusion. Potentiation of hippocampal-evoked response is induced in accumbens cells by HFS of the fimbria. This potentiation was blocked by SCH23390 or an NMDA antagonist (Floresco et al., 2001), and is associated with a transient increase in dopamine concentration in the accumbens. Thus, as in the dorsal striatum, a transient increase in dopamine concentration which is time-locked to the HFS-induced depolarization of nucleus accumbens neurons, is sufficient to facilitate subsequent hippocampal-evoked activity. The subsequent release of dopamine after induction of this facilitation does not appear to play a role (Floresco et al., 2001).

It is important to address whether the dopamine-dependent synaptic plasticity described could, in principle, underlie learning-related changes in the brain. The role of synaptic plasticity in normal reward-related learning has been investigated using intracranial self-stimulation (ICSS) as a model for reward-related learning, in which rats learn to press a lever repeatedly to electrically stimulate their own dopamine neurons in the substantia nigra. Using the same animals in which ICSS responding had been measured, Reynolds et al. (2001) then made in vivo intracellular recordings from striatal neurons, and measured responses to cortical afferents before and after a ICSS-like stimulation of the substantia nigra dopamine cells. Stimulation of the substantia nigra with behaviorally-reinforcing parameters induced potentiation of corticostriatal synapses. In addition, the degree of potentiation up to 10 min after the stimulus trains was correlated with the rate of learning of ICSS. Animals showing a greater degree of potentiation were correspondingly faster to reach criteria for ICSS, and vice versa. Potentiation was blocked in control animals administered a dopamine D1-like receptor antagonist (Reynolds et al., 2001). These findings suggest that stimulation of the substantia nigra may positively reinforce behavior by dopamine D1 receptor-dependent potentiation of cortical inputs to the striatum.

In summary dopamine-dependent synaptic plasticity is a potential cellular mechanism for reward-related learning in the striatum. Dopamine pulses produced by pressure-ejection or substantia nigra stimulation may mimic the effects of natural reward. The correlation of degree of synaptic change with rate of learning with ICSS is highly suggestive of a relationship between reward-related learning and dopamine-dependent synaptic plasticity in the striatum.

3.4. STRUCTURAL PLASTICITY

In parallel to the discovery of the long-term actions of dopamine on the sensitivity of the corticostriatal pathway, there have been a series of studies, which suggest that in the striatum, as in the hippocampus, the changes in synaptic strength have structural consequences. The earliest of these is probably the study of spine numbers in the neostriatum after destruction of dopamine input (Ingham et al., 1989). After a 6-OHDA lesion it seemed that there were fewer spines on individual dendrites in the striatum. The effect was visible as soon as the damage became stable at three weeks after the lesion and was still present one year later. But the lesion does not involve glutamatergic neurones. So perhaps the spines that are lost may have had dopamine synapses on them. That too seems unlikely since we estimated earlier that only about 11% of spines would have a dopamine

synapse on them and approximately 20% of the spines were lost after removing dopamine. Further work from the same group has shown that a parallel loss of asymmetric synapses occurs at three weeks after the 6-OHDA lesion (Ingham et al., 1998). More recently the group have documented an even more extensive loss of spines in the post mortem brains of patients with Parkinson's disease (Ingham et al., 2002). So the loss of dopamine seems to cause the loss of spines and their associated asymmetric synapses. Is this a kind of long term depression of synaptic structure?

Of course, the more interesting case is that of the formation of new spines, or the modification of the synaptic structure, after strengthening of the asymmetric synapses on them. Direct evidence relevant to that idea is lacking, but there is a tantalizing hint that something of the sort may be happening. Robinson and colleagues (Robinson and Kolb, 1999) show a rise in spine number in conditions of an exposure to drugs of abuse that lead to behavioral sensitization to the substances. At present, there is no equivalent to the beautifully specific evidence that strengthened synapses are associated with new spine formation that have been carried out in the anatomically simpler hippocampal situation (Engert and Bonhoeffer, 1999) or even in the more accessible cortex of the rat (Grutzendler et al., 2002; Ottersen and Helm, 2002; Trachtenberg et al., 2002). The methodology being developed in several laboratories, which allows the study of corticostriatal systems in vitro, may lead to similar results being obtained in the striatum in the perhaps not too distant future.

4. SYNTHESIS AND CONCLUSIONS

Several lines of evidence indicate that the dopamine signal in the striatum is time-specific, but not spatially focussed at the synaptic level. Dopamine receptors are not only concentrated at the synaptic cleft of the dopaminergic synaptic contacts, but are also found in significant amounts at extrasynaptic locations, often postsynaptic to glutamatergic synapses of cortical or thalamic origin. This applies to both D1-like and D2-like receptor subtypes. Similarly, the dopamine transporter, which brings about termination of the dopamine signal by reuptake, is extrasynaptically located. This arrangement of receptors and transporters implies dopamine signaling is directed, in part, towards nearby glutamatergic synapses and involves significant overflow of dopamine into the extracellular space. Electrochemical measurements of dopamine overflow and diffusion appear to confirm this.

The dopamine released from a single synaptic site forms an expanding sphere of increased dopamine concentration, which collapses under the control of the dopamine reuptake mechanism. The precise dimensions of each sphere are on the order of micrometers. The density of dopaminergic and glutamatergic synapses is such that the average distance to the nearest dopaminergic synapse is on the order of one micrometer, and therefore well within its sphere of influence. The overlap from the nearby dopamine release sites is such that asynchronous firing of the dopamine cell population is likely to produce, by summation, a steady and more-or-less uniform distribution of dopamine concentration.

A loosely time-locked discharge of the dopamine cell population in response to an unexpected reward would produce a phasic increase in dopamine concentration throughout the striatum. The active dopamine reuptake mechanism terminates this increase within tens of milliseconds, Thus, the phasic, reward-related dopamine signal is a

pulse-like event which reaches all cells and synapses in the striatum almost simultaneously. A pause in firing, conversely, would produce an inverted pulse of decreased dopamine concentration.

The postsynaptic transduction of the dopamine signal, whether steady-state, pulse increase, or the pulse decrease in concentration, depends on the relative predominance of D1-like or D2-like dopamine receptors in the postsynaptic cell. The steady-state levels appear to be sufficient to activate both subtypes of receptor, as locally applied antagonists of either receptor subtype produce physiological effects. Two broad classes of postsynaptic effects can be identified: immediate, short-term effects which reverse rapidly, and longer-term effects which persist after the removal of the dopamine signal.

The immediate short-term effects of dopamine are mediated by voltage and receptor-operated channels. The effects depend on the membrane potential of the postsynaptic cell, and its recent history, because these variables determine the state of the channels modulated by dopamine. At hyperpolarized potentials, rapidly inactivating channels are available, and modulating them can have effects on the transition from hyperpolarized Down states to depolarized Up states. After prolonged periods in the depolarized states, these channels are inactivated. If a dopamine pulse occurs at this time the effects on noninactivating channels will predominate.

As a working hypothesis, we propose that a transient increase in dopamine concentration may interact with the postsynaptic cell in a state-dependent way to produce an effective 'sample and hold' mechanism. For example, in hyperpolarized neurons, increasing the inward rectifier current by D1 receptor activation holds cells hyperpolarized. In depolarized neurons, D1 receptor activation increases noninactivating calcium currents, and this favors maintained depolarization. The functional significance of such a mechanism may be to produce a short-term increase in the gain of selected corticostriatal pathway circuits that may facilitate approach to rewarding stimuli. On a longer time scale, a transient increase in dopamine concentration may also lead to activity-dependent potentiation of active inputs on active cells. The sample and hold effect ensures that new states do not intervene while the activity-dependent potentiation is being induced. Together, these mechanisms favor activation by incentive stimuli and strengthening by positive reinforcement of selected corticostriatal pathway circuits.

These mechanisms play a crucial role in the normal behavioral regulation of the organism. They also become disordered by abnormal activation of the dopamine pathways, as occurs with psychostimulant drug use; or by underactivation, as occurs with neuroleptics. In the extreme case, degeneration of the dopamine neurons may lead to a persistent hypodopaminergic state, as in Parkinson's disease. In a simple-minded way, the pathophysiological changes in these disorders may be seen as exaggerated forms of the normal functions, leading to inappropriate amplification of behaviors (as in addiction), or chronic loss of the ability to produce behavior (as in Parkinson's disease).

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CHAPTER V

Motor function(s) of the nigrostriatal dopamine system: studies of lesions and behavior

STEPHEN B. DUNNETT

ABSTRACT

Ever since the initial discovery of the system, damage to the nigrostriatal dopamine system has been associated with motor impairments, akinesia in animals and Parkinson's disease in man. The present review focuses on the experimental techniques involving the use of pharmacological and lesion manipulations in experimental animals, particularly in rats, to explore the role of dopamine neurones in normal motor behavior and a detailed analysis of its role in the disability, plasticity and recovery of functions. Experiments based on the novel toxins and the use of the genetic models are reviewed and compared with the classical neurochemical lesions. Together, these studies indicate that dopamine neurones are not simply permissive – allowing normal motor behavior to be expressed – but are involved in the selection and the initiation of appropriate actions, and in establishing and maintaining motor skills and habits.

KEY WORDS: Motor systems; behaviour; 6-hydroxydopamine; nigrostriatal lesions; rats; monkeys; transgenic mice.

1. INTRODUCTION: THE CLASSICAL MODELS

The recognition of motor functions of the nigrostriatal dopamine system went hand in hand with the original identification of dopamine itself as a neurotransmitter. In his pioneering experiments to manipulate catecholamine (dopamine and noradrenaline) synthesis pharmacologically, Carlsson (1959) found that blocking catecholamine storage with reserpine or blocking de novo synthesis with α-methyl tyrosine produces a profound akinesia in experimental rats and rabbits. Realizing that the motor symptoms were more closely correlated with the depletion of dopamine from the striatum than with the depletion of noradrenaline from its primary projection areas in cortex, hippocampus or hypothalamus, combined with the fact that noradrenaline exists at only very low levels in the striatum, led him to propose that dopamine exists in the brain not only as a precursor to the known neurotransmitter noradrenaline, but may act as a neurotransmitter in its own right. Moreover, the association of motor deficits with dopamine depletion in the striatum implied that a separate dopamine system in the basal ganglia is importantly involved in motor activation.

A second fundamental insight arose from the same data. The similarities of the experimental akinesia produced by reserpine or α-methyl tyrosine to the bradykinesia of

Parkinson's disease (PD) provided the first suggestion that PD might itself be attributable to dopamine loss from the striatum (the caudate nucleus and/or putamen in humans). This suggestion was soon confirmed in postmortem biochemical analyses (Ehringer and Hornykiewicz, 1960). Moreover, Carlson's demonstration that the reserpine-induced motor syndrome can be reversed in experimental rabbits by the administration of the dopamine precursor dihydroxyphenylalanine (DOPA, Fig. 1) (Carlsson et al., 1957) naturally led to the discovery that the DOPA could similarly alleviate parkinsonian dyskinesia in patients (Birkmayer and Hornykiewicz, 1961). These early trials produced significant side effects, such as nausea due to peripheral actions of the drug that limited its therapeutic usefulness. However, considerable improvement in therapeutic specificity has been achieved using the active levo-isomer, L-DOPA, in combination with a peripheral inhibitor, carbidopa (Cotzias et al., 1967), and this has become the mainstay of a practical therapy for PD, notwithstanding additional problems of fluctuation, wearing off of efficacy and the dyskinetic side effects that can develop with long-term treatment and progression of the disease (Marsden and Parkes, 1976, 1977).

It should not be thought that the flow of information has been unidirectional, from the lab to the clinic; models of PD have been one of the most widely studied systems for the analysis of the relationship of structure to function in the CNS in animals, and Marsden (1992) has proposed that PD provides a prototypical system for studying the relationship of the basal ganglia organization and the normal motor function in man.

Biochemical analyses alone were insufficient to identify and map the critical neuronal population and their associated pathways, and it became necessary to wait for the

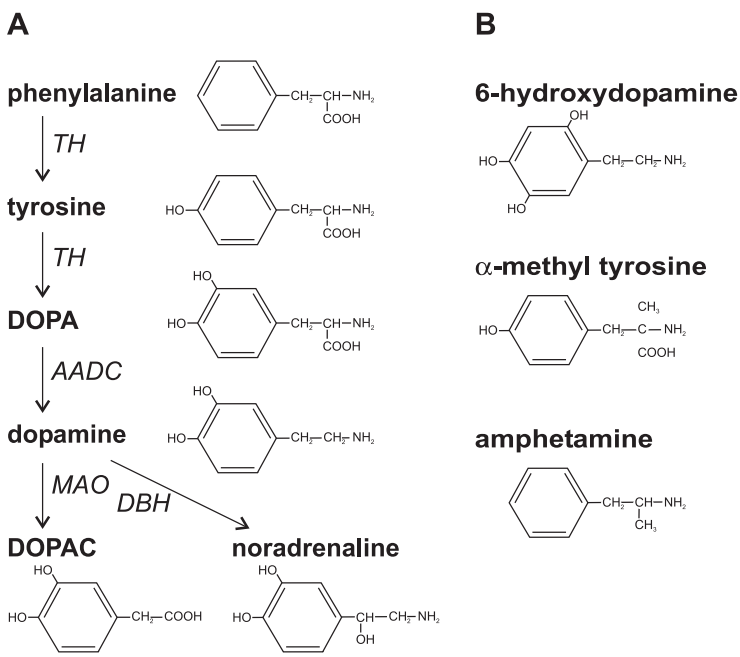

Fig. 1. **A.** Chemical structure of key molecules involved in the key steps in intracerebral synthesis and metabolism of dopamine. The successive steps are regulated by the enzymes tyrosine hydroxylase (TH), aromatic amino acid decarboxylase (AADC), monoamine oxidase (MAO) and dopamine-β-hydroxylase (DBH). **B.** Structure of key toxins and other drugs acting on dopamine neurones, including 6-hydroxydopamine (6-OHDA), α-methyl tyrosine, and amphetamine. For further details see Iversen and Iversen (1981) or Cooper et al. (1996).

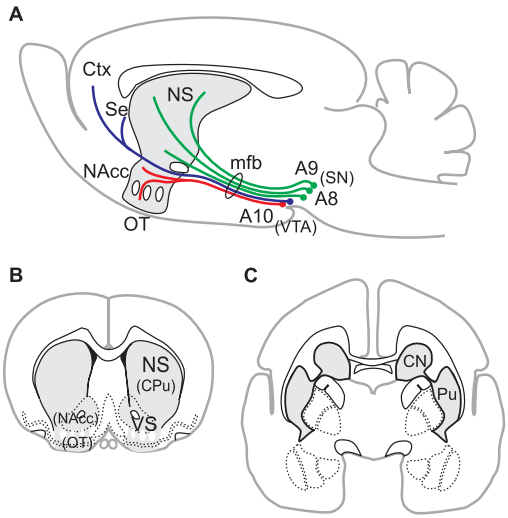

Fig. 2. **A.** Forebrain dopamine projection system in rodents and primates. The nigrostriatal pathway projects from the A8 and A9 groups of the substantia nigra (SN) via the medial forebrain bundle (mfb) to the neostriatum (NS). The mesocorticolimbic pathway projects from the more medially located A10 cell group of the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) and olfactory tubercle (OT) of the ventral striatum (VS) and limbic forebrain areas including prefrontal cortex (Ctx), septum (Se) and amygdala (A). **B.** Striatal projection areas in the rodent brain are divided into the more dorsal neostriatum, and ventral striatum. **C.** In the primate brain, including human and illustrated for the marmoset, the neostriatum is divided by the fibers of the internal capsule into caudate nucleus (CN) and putamen (Pu). Correspondingly, the neostriatum of rats is sometimes designated the 'caudate-putamen' (CPu) complex.

introduction of catecholamine fluorescence (Falck et al., 1962), the mapping of the brainstem catecholamine cell groups (Dahlström and Fuxe, 1964) and the use of stereotaxic lesions of the pathway (Ungerstedt, 1971c) to map out the identity of the critical nigrostriatal substrate with dopamine cell bodies in the A8–A10 groups of the ventral mesencephalon projecting in a broad swath of projections, via the medial forebrain bundle to the striatum (Fig. 2, Moore and Bloom, 1978; Ungerstedt, 1971c). With the further development of refined anatomical mapping techniques involving modifications of the fluorescence reaction and the introduction of immunohistochemical methods, the precise organization of the mesencephalic dopamine pathways soon followed, and we now have detailed descriptive understanding of the topography and organization of projections from the discrete nuclear groups in the ventral mesencephalon to the neostriatum, ventral striatum and mesocortical areas (Lindvall and Björklund, 1974; Björklund and Lindvall, 1986). A summary of the key pathways relevant to the present functional discussion is shown in Fig. 2, and a detailed review has been presented in an earlier volume, Vol. 2, in this Handbook series (Björklund and Lindvall, 1984).

2. SPONTANEOUS MOTOR EFFECTS OF DOPAMINERGIC DRUGS

2.1. ANTAGONISTS: AKINESIA AND CATALEPSY

The first and the most direct way to identify a function associated with an activity in the nigrostriatal dopamine system is to manipulate dopamine transmission pharmacologically,

as first used so powerfully by Carlsson. One of the reasons behind the extensive study of the dopamine system is the availability of powerful drugs to manipulate catecholamine synthesis, storage, release, receptor binding and re-uptake, which have correspondingly powerful effects on behavior (Cooper et al., 1996; Feldman et al., 1997). Since dopamine is not only a precursor to noradrenaline and adrenaline in adrenergic neurones, but also a neurotransmitter in its own right in neurones that do not express the converting enzyme dopamine-β-hydroxylase, Carlsson's studies using drugs that blocked synthesis (α-methyl tyrosine) and storage (reserpine) would have affected both the dopamine and the noradrenaline release, so that he was dependent upon correlations of behavioral changes with biochemical depletions to infer transmitter specificity. As an alternative, though, we can now use a wide range of antagonists of the dopamine receptor to infer specificity of effects either to dopamine (in contrast to other catecholamines) or to specific subclasses of the dopamine receptor (in particular D1- vs. D2-like receptors), although it remains the case that pharmacological compounds are not, even now, available with the specificity to block completely and selectively the individual (D1–D5) receptors that have been determined molecularly (Gingrich and Caron, 1993; Feldman et al., 1997).

The largest class of drugs that block dopamine receptors are the neuroleptics, first developed for their sedative action in schizophrenia. Some, including phenothiazines such as chlorpromazine, are antagonists at both noradrenaline and dopamine receptors, but others such as haloperidol, fluphenazine, pimozide and spiroperidol are relatively selective for the dopamine synapse (Iversen and Iversen, 1981). These drugs are used clinically for their tranquilizing effects on patients. In the experimental animals, they cause hypokinesia or akinesia (measured as a reduction in locomotor activity in rats) and catalepsy (the adoption of abnormal posture and resistance to movement). The inactivity associated with dopamine receptor blockade appears to involve an active resistance to movement since the animals brace themselves against attempts at displacement (Mason, 1984), quite distinct from the more flaccid responses to other classes of akinetic compounds (Costall and Naylor, 1973; De Ryck et al., 1980). Moreover, they can move when activated with an appropriate stimulus (Feldman and Lewis, 1962), akin to the paradoxical kinesis occasionally reported in PD patients (Sacks, 1973). Catalepsy appears to be mediated at the level of dopamine terminals in the striatum, since catalepsy can be induced by bilateral injection of dopamine antagonists in the striatum (Fletcher and Starr, 1988; Klockgether et al., 1988; Iakimovskii, 1993; Meyer et al., 1993) and catalepsy associated with peripheral delivery of haloperidol is blocked by lesions of postsynaptic neurones in the neostriatum (Sanberg, 1980; Al-Khatib et al., 1989). Both D1-like and D2-like receptors appear to be involved (Fletcher and Starr, 1988; Wanibuchi and Usuda, 1990).

The dopamine receptors are not passive transducers of transmitter signals, but adapt dynamically to changes in their activation. Dopamine system plasticity will be developed in more detail in the context of responses to lesions, but it can be noted that chronic administration of neuroleptics, resulting in a reduction in locomotor activity when the drug is active, in turn results in the development of receptor 'supersensitivity'. Once the drug is washed out of the system, the receptors remain above-normally responsive as manifested by behavioral hyperactivity in the undrugged animal, an even more hyperactive response when the animals are administered low doses of a receptor agonist, and an increased binding of receptor ligands when studied by imaging or receptor autoradiography (Iversen and Iversen, 1981; Huang et al., 1997a; Tarazi et al., 1997; Besret et al., 2000).

2.2. AGONISTS: HYPERACTIVITY AND STEREOTYPY

The converse of akinesia and rigidity to dopamine receptor blockade is the hyperactivity and stereotypy that results from dopaminergic stimulation. Again a wide variety of drugs are available with varying degrees of specificities, the most powerful and widely studied in experimental animals being the indirect and direct agonists, amphetamine and apomorphine, respectively.

Amphetamine exerts its effects by stimulating vesicular release of catecholamines and blocking its re-uptake, which together increase the quantity and prolong the duration of the endogenously released transmitter available for binding at the receptor (Teitelbaum and Stellar, 1954). Following peripheral injection at an effective dose, amphetamine induces a marked increase in locomotor activity which lasts for a 4–5 h duration of the drug action (see Fig. 3A) (Malmo, 1959; Taylor and Snyder, 1970a; Creese and Iversen, 1972; Scheel-Kruger and Jonas, 1973). A variety of different tests and apparatus are available for monitoring the activities of rats and mice, from the very simple cages with one or two photocell beams in which the total count of beam breaks is automatically recorded, to the complex radar tracking and movement analysis devices (Robbins, 1977). However, for the present purpose, they all reveal rather similar results: with ascending doses of amphetamine there is an 'inverse U' relationship, whereby increasing doses yield progressive increases in locomotor activity up to a peak dose, around 1.5 mg/kg i.p. in

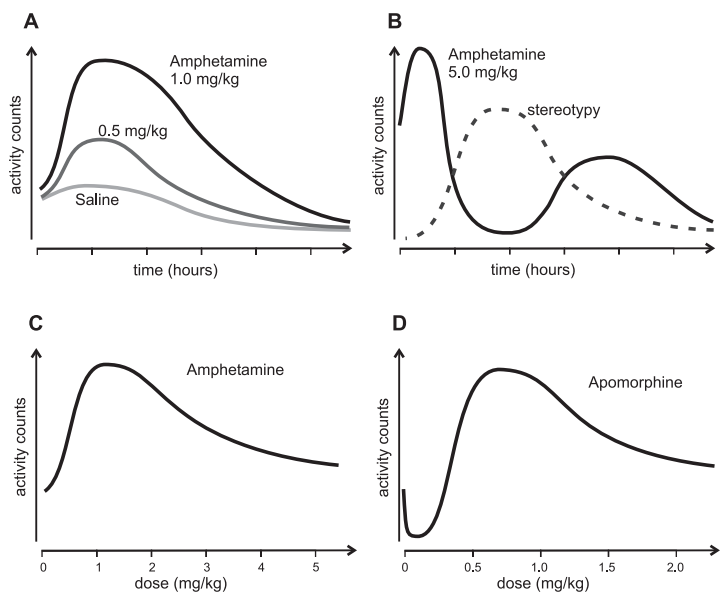

Fig. 3. Locomotor hyperactivity to drugs activating forebrain dopamine systems (schematic illustration of response). **A.** Moderate doses of amphetamine induce progressive dose-dependent increases in counts recorded in automated activity chambers. **B.** At higher dose, amphetamine induces stereotypy which competes with the expression of locomotion resulting in a suppression of activity counts at peak dose. **C.** Competing stereotypy yields an 'inverse U' function in the dose response curve (total activity over 2 h test). **D.** The dopamine receptor agonist apomorphine also increases activity at moderate doses which is blocked by competing stereotypy at high dose, but this drug also inhibits activity at the lowest doses, believed to be due to selective action at presynaptic autoreceptors (see text).

rats and mice; with further increase in dose, the total activity declines again until falling below the normal baseline above about 5 mg/kg (see Fig. 3C). At high doses, a biphasic response is seen, whereby the animals are hyperactive over 10–20 min in the early period of the test as the drug slowly penetrates into the brain. The activity is suppressed in the middle period of the test, and then rises again after 3–4 h as concentration in the CNS dissipates (see Fig. 3B).

Although amphetamine activates both the dopamine and the noradrenaline systems, the locomotor activation appears to be predominantly due to action at dopaminergic rather than noradrenergic terminals. One line of argument has related the response to different isomers of amphetamine which have different effects on dopamine vs. noradrenaline release and uptake, although the interpretation of these results has proved problematic (Taylor and Snyder, 1970b; Bunney et al., 1975). Clearer results come from central microinjection and lesion studies. Thus, the response to peripheral amphetamines is mimicked by injection of dopamine or amphetamine directly into the striatum, in particular the ventral striatum (Pijnenburg and van Rossum, 1973; Pijnenburg et al., 1976; Makanjuola et al., 2003). Conversely the response is blocked by inhibition of the dopamine receptors by central injection of haloperidol (Pijnenburg et al., 1975) and by lesions that produce selective depletions of dopamine vs. noradrenaline (Smith et al., 1973) or ones that target the dopaminergic terminals in the nucleus accumbens/ventral striatum (Kelly et al., 1975; Kelly and Iversen, 1976).

At higher doses, amphetamine induces a pattern of abnormal behaviors known as 'stereotypy' (Randrup and Munkvad, 1967, 1975). These are short response sequences repeated over and over, such as abnormal mouth movements, head-down sniffing at a fixed location, short components of a grooming sequence or gnawing of a cage bar. The individual components of the movements come from the normal repertoire but are repeated in a truncated form, without achieving any goal. Amphetamine abuse causes similar stereotyped movements and thought patterns in human drug takers (Randrup and Munkvad, 1967). Stereotypy is much more difficult to measure objectively in animals than activity, but a number of well-defined observational rating scales have been devised (Creese and Iversen, 1973; Fray et al., 1980; Molloy and Waddington, 1987).

Activity and stereotypy have to be considered together when interpreting the biphasic dose–response curves in a locomotor activity. Stereotypy begins appearing at the peak dose for locomotor activation, and since stereotypy in a fixed location is incompatible with locomotion over wide areas of a test arena, the decline of activity at high doses and in the middle periods of a test is most readily interpreted as competition between the two classes of response, stereotypy displacing locomotor activity. Nevertheless, they need not be considered as qualitatively different responses to the drug. Rather, in what has become known as the Lyon–Robbins hypothesis, increasing doses of amphetamine progressively increase the likelihood and the frequency of expressing any behavior; and at the highest doses the shortest and simplest response elements come to dominate in the competition as the only components reaching overt expression in observed behavior (Lyon and Robbins, 1975). Stereotypies induced by amphetamine also seem to be mediated by dopaminergic rather than noradrenergic systems, since they are readily induced by direct receptor agonists, such as apomorphine (Randrup and Munkvad, 1974; Mason, 1984). Moreover, like the hyperactivity, stereotypy is similarly blocked by lesions of forebrain dopamine projections that spare the forebrain noradrenaline pathways (Creese and Iversen, 1973, 1975), although the primary locus appears to be more within

dorsal than ventral striatal sites (Creese and Iversen, 1974; Kelly et al., 1975). Consequently, selective lesions of the dorsal striatal terminals will eliminate the competing stereotypical responses, releasing a progressive increase of locomotor hyperactivity with increases of amphetamine, rather than a downturn at the higher doses (Joyce and Iversen, 1984).

A number of key differences have been described between the precise behavioral outcomes of indirect agonists, such as amphetamine, and the direct agonists, such as apomorphine, which may relate to differences in the loci of different subclasses of dopamine receptors. Thus, amphetamine appears to have a broader range of doses over which the drug induces locomotor hyperactivity, whereas apomorphine appears to induce competing stereotypies at lower doses in the range. Careful movement analyses highlight that the behavioral expression of stereotypies induced by the two classes of drug differ in ways that cannot be attributed to differences in dose–response, with amphetamine inducing more wide ranging and varied movements and apomorphine inducing the most hunched head-down sniffing and licking at a single location, although the precise pattern of expression can be due to a wide variety of internal and external factors (Szechtman et al., 1985, 1988). Lastly, and importantly, whereas amphetamines induced the biphasic dose–response effects on locomotor activity, as described above (moderate doses inducing hyperactivity, but high doses inducing in reductions of activity due to competition from stereotypical behaviors), apomorphine is associated with a tri-phasic response whereby at very low doses the rats exhibit an inhibition of activity (Fig. 3D; Ljungberg and Ungerstedt, 1976, 1977). This effect has been attributed to the action of the direct agonists at sensitive presynaptic 'autoreceptors' (Carlsson, 1975), an effect which would be less marked when dependent on activation by endogenous dopamine, released (and rapidly retaken up) following the administration of low doses of amphetamine.

3. BILATERAL NIGROSTRIATAL LESIONS IN RATS

3.1. THE 'LATERAL HYPOTHALAMIC' SYNDROME

The first lesion model disrupting the forebrain dopamine systems to be studied in detail was that involving electrolytic or radiofrequency lesions of the lateral hypothalamus. The 'lateral hypothalamic syndrome' was extensively studied in the 1950s and 1960s in the context of diencephalic motivational systems without realizing, at the time, that a large proportion of the 'sensorimotor' effects of those lesions was attributable not to disruption of intrinsic hypothalamic circuits, but to collateral damage of the forebrain dopamine axons ascending through the lateral hypothalamic area from midbrain to striatal and corticolimbic areas. A brief survey is appropriate because the models and tests developed in that context have had a significant influence on the subsequent development of behavioral analysis and our understanding of the function of the forebrain dopamine system.

With the early application of stereotaxic surgery to neurological studies in experimental animals, Anand and Brobeck (1951) first reported that bilateral lesions of small nuclei in the hypothalamus could disrupt feeding behavior – with medial hypothalamic lesions causing obesity and lateral lesions aphagia. In the latter syndrome, the lesioned animals would completely stop eating to the extent that if not maintained

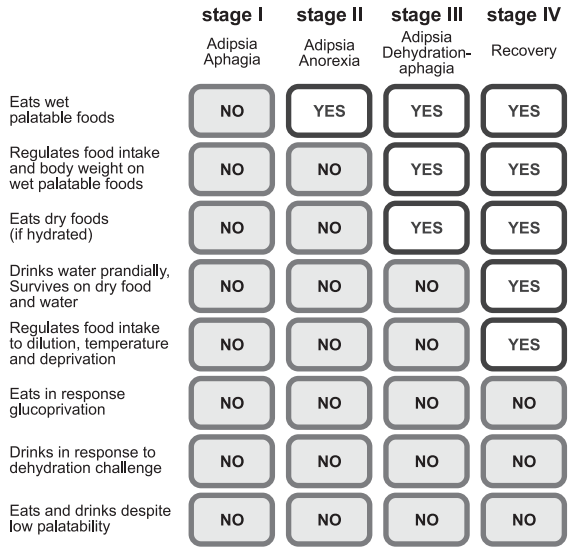

Fig. 4. Proposed stages of recovery from the profound regulatory impairments induced by lateral hypothalamic lesions, based on Teitelbaum and Epstein (1972). A similar profile of recovery is seen from the impairments induced by selective bilateral lesions of forebrain dopamine systems (see text).

by tube feeding, they would starve to death. It soon became apparent that these lesions produced not only aphagia, but also adipsia, hypoactivity or, *in extremis*, full akinesia and catalepsy (Teitelbaum and Stellar, 1954; Teitelbaum and Epstein, 1962a; Levitt and Teitelbaum, 1975). Although much attention was given to specific impairments in the regulation of food and water intake (possibly involving disruption of a discrete 'hunger' center in the brain), the syndrome was clearly much broader, involving a profound disruption of a wide range of voluntary, goal-directed or motivated behaviors (Levitt and Teitelbaum, 1975). Conversely, stimulation of the lateral hypothalamus induces a range of 'stimulus-bound' behaviors, in which the animals engage in complex specific behavioral routines associated with a variety of motivational states, such as eating or drinking, nest-building, gnawing on wood chips, or active exploration, sniffing and digging (Valenstein et al., 1968; Cox and Valenstein, 1969; Valenstein and Cox, 1970; Bachus and Valenstein, 1979).

One of the most remarkable features of the lateral hypothalamic lesion syndrome is that, when the animals have been kept alive by rehydration and tube feeding, and although they had been so debilitated in the days after surgery and had not eaten and drunk spontaneously, most animals eventually show a substantial degree of recovery of the ability to eat, drink and maintain themselves without additional care (Teitelbaum and Stellar, 1954). The recovery takes place in a regular sequence of stages involving first a willingness to sample moist and palatable foods, then spontaneous drinking, if associated with eating dry but palatable foods, and finally an ability to maintain themselves on normal lab chow and water (Fig. 4). However, the 'recovered' animals never recover the ability to regulate food and water intake in response to the challenges of the internal physiological state, such as glyoprivation, intracellular or extracellular dehydration

(Teitelbaum and Stellar, 1954; Teitelbaum and Epstein, 1962b). Theories proliferated in terms of complex systems of the hypothalamus involved in the control of motivational behaviors, with much discussion of whether there was need to infer multiple distinct circuits for the control of different motivational states, such as hunger, thirst, sexual drive, exploration, play, fear and anxiety, or whether by contrast there was some integrating principle that could bring these components together into a common unifying process such as 'activation' or 'arousal' (Valenstein et al., 1970).

At the same time, a detailed search for anatomical substrates for the distinct circuitries that underlay motivational systems of the hypothalamus yielded speculation that disruption of the pathways of the medial forebrain bundle may contribute to the syndrome (Morgane, 1961; Lyon et al., 1968). In particular, Grossman undertook a series of knife-cut lesions at various levels of the ascending noradrenaline, serotonin and dopamine pathways passing through the hypothalamus, suggesting that a disruption outside the hypothalamus itself can induce most components of the lateral hypothalamic system (Grossman 1971; Alheid et al., 1977). Conversely, cell-specific lesions within the hypothalamus which spare axons of passage produce deficits in specific aspects of food and water intake regulation but do not induce the profound aphagia, adipsia, akinesia or neglect associated with the classic syndrome (Grossman et al., 1978; Winn et al., 1984; Dunnett et al., 1985). These studies gave credence to the emerging view that although the hypothalamic circuits may mediate specific homeostatic regulatory processes (such as those involved in the osmotic regulation of thirst, glucostatic and other specific nutritive factors regulating hunger, and temperature regulation), other more general motivational processes (such as those associated with the overlapping psychological concepts of activation, arousal, drive and reward) probably relate to more diffusely organized forebrain systems collocated with, or passing through, the lateral hypothalamic area.

Often, it was the introduction of new techniques – for the selective lesion of hypothalamic neurones on the one hand and of catecholamine fibers of passage on the other – that provided the turning point in the debate. To address one side of the dissociation, cell-specific lesions within the hypothalamus which spare axons of passage produce deficits in specific aspects of food and water intake regulation but do not induce the profound aphagia, adipsia, akinesia or neglect associated with the classic syndrome (Grossman et al., 1978; Winn et al., 1984; Dunnett et al., 1985). Conversely, many of the components of the lateral hypothalamic syndrome are mimicked by lesions selective to the ascending nigrostriatal dopamine system. The toxin 6-hydroxydopamine (6-OHDA) was first described in 1968 for selective lesions of catecholamine neurones in both the peripheral sympathetic systems (Thoenen and Tranzer, 1968; Tranzer and Thoenen, 1968) and in the forebrain (Ungerstedt, 1968; Bloom et al., 1969; Uretsky and Iversen, 1969). In the very initial studies, it was noticed that these lesions produced profound motor deficits, including hypokinesia and the disruption of food and water intake (Evetts et al., 1969; Ungerstedt, 1970, 1971a). It was Ungerstedt who first drew explicit attention to the similarities between the 6-OHDA and lateral hypothalamic lesion syndromes, leading him to hypothesize that much of that the classic literature may be directly attributable to disruption of not only intrinsic hypothalamic circuits but also of the ascending dopamine fibers of passage (Ungerstedt, 1970). This hypothesis has been amply confirmed in the subsequent studies, reviewed in the following sections.

3.2. INTRAVENTRICULAR/BILATERAL NIGROSTRIATAL 6-OHDA LESION SYNDROME

Identification of the synthetic molecule 6-hydroxydopamine as a catecholamine-selective toxin has revolutionized the study of dopamine system function and the development of animal models of a variety of disease states, most notably Parkinsonism. 6-OHDA is a structural analog of dopamine with an additional hydroxyl group at position 6 of the benzene ring (see Fig. 1B). The molecule is an effective 'Trojan horse' – it is selectively taken up into both the dopamine and the noradrenaline neurones by the active transmitter re-uptake channels and accumulated intraneuronally. 6-OHDA is highly electroactive, and rapidly oxidizes to produce both hydrogen peroxide and free radicals, which are highly cytotoxic, in particular in catecholamine neurones (Schwarting and Huston, 1996b; Dunnett and Björklund, 1999). It does not cross the blood-brain barrier, so it has to be administered intracerebrally in adult rats (Ungerstedt, 1968; Jolicoeur and Rivest, 1992), although peripheral administration is effective in neonatal rats before the barrier has formed (Breese and Traylor, 1971; see Section 3.4). Even though 6-OHDA is toxic against all catecholamine neurones in vitro, its potency and selectivity can be enhanced by both pharmacological and pharmacodynamic variations in its administration. In the neonatal animal, variations in the dosing regime and route of delivery can achieve marked differences in the pattern of toxicity (see Section 3.4). In adult rats, selective lesions of the telecephalic noradrenaline projection are promoted by making injections into the ventral mesencephalon at a site where the noradrenergic dorsal bundle separates in a loop upwards from other forebrain catecholamine fiber systems and dorsal to the location of the dopamine cells (Ungerstedt, 1971c; Moore and Bloom, 1979; Mason, 1984). Conversely, dopamine can be targeted selectively by pretreatment of the animals with the noradrenergic uptake blocker des-methyl-imipramine (DMI) prior to injection of 6-OHDA into the common medial forebrain bundle (Ungerstedt, 1971c; Moore and Bloom, 1978; Mason, 1984). Toxicity against either system can be further promoted by treatment with the monoamine oxidase inhibitor pargyline, which prolongs the period of 6-OHDA availability for uptake prior to its oxidation by endogenous enzymes.

After Ungerstedt's (1970) lead, Stricker and Zigmond provided the first detailed descriptions of the behavioral consequences of forebrain dopamine depletion, using a bilateral lesion model based on injection of 6-OHDA into the lateral ventricles of adult rats. In the first study (Zigmond and Stricker, 1972), 6-OHDA lesions were seen to induce rather mild reductions in food intake and only a modest body weight decline that recovered rapidly. However, it was noted that the 6-OHDA injections, while producing virtually complete loss of the forebrain noradrenaline, only depleted dopamine by about 62%, whereas lateral hypothalamic lesions can produce up to 95% depletions. Therefore, in a second group, the animals were pretreated with pargyline prior to 6-OHDA injection, which increased the striatal dopamine depletions to 96% and now resulted in profound and substantial weight loss (Zigmond and Stricker, 1972). In a more detailed analysis, in addition to the body weight loss and aphagia, rats with intraventricular 6-OHDA lesions were also seen to exhibit adipsia, lack of grooming, piloerection, irritability and postural abnormalities, were profoundly cataleptic, and showed impaired 'sensorimotor integration' as seen by a failure to respond to olfactory, tactile and visual stimuli (Zigmond and Stricker, 1973). Nevertheless, in spite of their initial debilities, most rats still recovered over a period of days and weeks to the point where they could eventually maintain themselves on dry food and water (Fig. 5), the recovery progressing through a similar

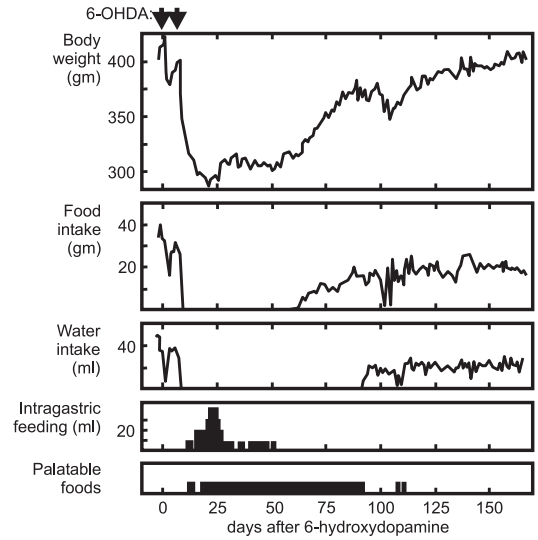

Fig. 5. Recovery in regulatory deficits after forebrain dopamine depletion. (Redrawn from Zigmond and Sticker (1973), with permission.)

series of stages to that which had previously been described for lateral hypothalamic lesions. The compensatory mechanisms by which dopamine neurones adapt as a substrate for recovery of function, has become a major topic for research in its own right (see Section 3.3).

Subsequent studies have characterized each of these components of the bilateral 6-OHDA lesions syndrome in greater detail. In particular, Marshall developed a range of detailed neurological tests to evaluate the sensory and motor capacities of the animals, revealing a profound bilateral neglect of multimodal sensory stimuli. Thus, the bilaterally lesioned animals fail to orient and respond to lateralized visual, auditory, olfactory or tactile stimuli (Marshall et al., 1974), similar to that seen after lateral hypothalamic damage (Marshall and Teitelbaum, 1974). Like the regulatory impairments, sensory neglect will also recover with time, if the animals are maintained by tube feeding. This is manifested as a gradual lowering of the threshold for responding, and progressing in a regular sequence from the rostral to the caudal levels of the body surface (Marshall et al., 1971), reminiscent of the parallel organized stages of recovery from the regulatory impairments.

Nevertheless the two syndromes are not identical and the differences are informative. On the one hand, both types of lesions induce profound aphagia, adipsia and akinesia, a failure to engage in a broad range of motivated behaviors, neglect of stimuli in all sensory modalities, and recovery in regular stages. On the other hand, animals with lateral hypothalamic lesions also induce somnolence according to both behavioral and electroencephalographic indices (Levitt and Teitelbaum, 1975), and exhibit impaired thermoregulation, which are not the major components of the nigrostriatal syndrome. Moreover, as noted even in the early studies by Zigmond and Stricker (1972), it requires a considerably greater depletion of dopamine with 6-OHDA to produce a comparable deficit to that seen in animals with lateral hypothalamic lesions. Together, these data suggest that the classical lateral hypothalamic syndrome comprises an intrinsic disruption

of a variety of local hypothalamic neural circuits for the regulation of specific physiological systems (such as cellular water, ion and glucose balance, thermoregulation, etc.), alongside collateral damage of the ascending dopamine pathways mediating a distinct motivational function. However, these should not be considered independent processes, because a major output of the lateral hypothalamus projects to the substantia nigra (Arbuthnott et al., 1976), providing a pathway whereby the outputs from specific regulatory hypothalamic systems provide a significant input into the general motivation/activation system of the forebrain, which is considered to be subserved by the ascending dopamine pathways. The recognition of multiple components to the classical syndrome highlights the futility of early discussions seeking to identify the common underlying deficit – whether a motor disorder, sensorimotor neglect, or disruption of general or specific motivations processes – but highlights the need to identify the separate functional contributions of those components. Thus, we shall return below, repeatedly, to the central question of this chapter, viz. What is the functional role of forebrain dopamine systems in the organization of behavior in man and other experimental mammals?

3.3. PLASTICITY AND RECOVERY OF FUNCTION

An important feature in Zigmond's studies on recovery of function was the observation that although behavioral recovery progressed through a series of stages, the recovered rats still exhibited very extensive depletions of the striatal dopamine levels. It might be that other systems of the brain substituted for the lost dopamine connection. Alternatively, even the 'best' lesions are never complete and as little as 1–5% of spared dopaminergic terminals may retain the capacity to compensate. Evidence in favor of the latter position was suggested by the observation that α-methyl tyrosine, even at very low doses which have no detectable effect on normal rats, will immediately reinstate the full acute lesion syndrome in rats that have apparently fully recovered from the intraventricular 6-OHDA syndrome (Zigmond and Stricker, 1973).

An important development from the early observations of recovery has been the search to identify the mechanism(s) that underlie compensation and plasticity. The forebrain nigrostriatal dopamine neurones have become the most studied system in the brain for the capacity of neurones to compensate biochemically and physiologically in response to damage. Thus, in a theoretical review, Stricker and Zigmond (1976) proposed a series of potential changes pre- and post-synaptically whereby the spared neurones in an injured system could upregulate their activity to regain functional control over their targets (see Fig. 6). Most of these components have subsequently been demonstrated experimentally:

Changes in dopamine synthesis. Catecholamine synthesis is regulated by tyrosine hydroxylase (TH) activity. A five-fold increase in TH activity and increased synthesis of noradrenaline in spared noradrenergic neurones after intraventricular 6-OHDA lesions was first reported in the hippocampus (Acheson et al., 1980), but this was soon followed by similar observations of increased production of TH in the striatum after similar global lesions (Zigmond et al., 1984). Moreover, there is a direct correlation between the degree of dopamine depletion and the extent of upregulation of the TH activity in residual terminals, being increased greater than six-fold in the most depleted cases (Zigmond et al., 1984).

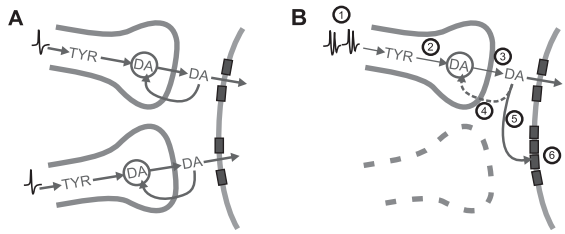

Fig. 6. Proposed hypothetical mechanisms of pre- and post-synaptic plasticity that might underlie recovery of function following partial lesions of the nigrostriatal dopamine neurones. **A.** Schematic illustration of two intact dopamine terminals making synaptic contact with a post-synaptic striatal neurone. **B.** Following partial lesion, spared terminals up-regulate to restore normal levels of postsynaptic activation by a combination of processes that include (1) increased firing, (2) increased dopamine synthesis, (3) increased dopamine release, (4) reduced dopamine uptake, (5) diffusion to deafferented synapses, and (6) supersensitivity of all post-synaptic synapses. (Redrawn from Zigmond and Sticker (1984), with permission.)

Changes in dopamine release and re-uptake. Dopamine release – as estimated by the postmortem ratio of the primary dopamine metabolite dihydroxyphenylacetic acid (DOPAC) to dopamine – has been seen to increase by about 250% in the striatum after an intraventricular 6-OHDA (Zigmond et al., 1984). At the same time, the increased number of transmitter molecules that are released are available longer as a result of a reduction of the pre-synaptic re-uptake as measured by a marked decline in the incorporation of tritiated dopamine into striatal synaptosomes (Zigmond et al., 1984). A more direct measure of dopamine release can be provided by in vivo dialysis. Thus, with progressively larger lesions, extracellular dopamine remains relatively constant at normal levels up to about 80–90% neuronal loss, reflected by a progressively greater release of dopamine from the terminals of each remaining neurone, and only at an even higher level of cell loss do the residual neurones fail to compensate (Abercrombie et al., 1990; Castañeda et al., 1990; Robinson et al., 1990).

Changes in neuronal activity. Dopamine neurones spared by a lesion also compensate by changes in their electrophysiological properties. Thus, spared nigrostriatal dopamine neurones increase their rates of firing and increase in burst firing activity (Hollerman and Grace, 1990), which together contribute to the increased levels of total dopamine release as well as the amount of dopamine release per action potential (Stachowiak et al., 1987).

Changes in post-synaptic response. When they lose inputs, post-synaptic receptors increase in sensitivity, resulting in an increased response to signal input-receptor 'supersensitivity'. This effect was first described in the context of behavioral responses to unilateral lesions (Section 4.3), but the effect can also be seen in a markedly hyperactive response induced by low doses of dopamine agonists in animals with bilateral forebrain dopamine lesions (Arnt, 1985; Breese et al., 1985b). Studies of the receptor binding indicate that dopamine receptor supersensitivity relates to increase in the density of dopamine receptors (Creese et al., 1977; Creese and Snyder, 1979), particularly of the D2 class (Arnt, 1985; Breese et al., 1985b; Moy et al., 1997). Nevertheless, receptor changes in adult animals with bilateral lesions can be subtle or not found at all (Duncan et al., 1987; Savasta et al., 1988; Reader and Dewar, 1999). This may be explained in part by extensive pre-synaptic compensation in dopamine release (Abercrombie et al., 1990), so that in animals with unilateral lesions, post-synaptic changes are only seen when the level of denervation is relatively high (Hefti et al., 1980), suggesting a required level of depletion

with bilateral lesions that is extremely debilitating to the animals and slow to recover (Zigmond and Stricker, 1973).

Anatomical sprouting. The traditional form of neuronal plasticity – for which there is in fact rather little evidence after nigrostriatal lesions – is sprouting, whether regenerative (i.e. grow back of damaged axons to their old targets) or collateral (i.e. growth of spared terminals to fill vacated adjacent spaces; Raisman and Field, 1973; Gage et al., 1982; Reader and Dewar, 1999; Hirsch, 2000). Although the nigrostriatal projection is predominantly ipsilateral, there is a small crossed projection from the contralateral substantia nigra (Fallon and Loughlin, 1982; Gerfen et al., 1982). Based on an increased retrograde labeling of nigral neurones on the opposite side after injections of tracers into the striatum after a large unilateral lesion of the ipsilateral nigrostriatal bundle, Pritzel and colleagues (1983) first suggested that terminals from the crossed connection could undergo local collateral sprouting to reinnervate the acutely denervated striatum, which may underlie the recovery of sensorimotor deficits exhibited by the animals. Certainly, again, dopaminergic fibers of the ipsilateral projection have a clear capacity for regenerative sprouting from adjacent spared terminals into a partially denervated striatum, in particular when given a strong stimulus as provided by an adrenal graft (Bohn et al., 1987; Hansen et al., 1995). Recently, several studies have provided a more detailed description of the appearance of growth cones and the expansion of the terminal arbour network over a period of 1–4 months associated with spared dopamine neurones on the ipsilateral side also (Blanchard et al., 1996; Liberatore et al., 1999; Finkelstein et al., 2000), a process that is regulated both by post-synaptic receptors and by glia activated by the lesion (Batchelor et al., 1999; Parish et al., 2002; Tripanichkul et al., 2003).

Putting these various components together, Zigmond and colleagues have proposed a scheme to describe the level of deficit and recovery after nigrostriatal lesion, whereby progressively more compensatory mechanisms are recruited depending on the level of initial denervation, and which will influence the precise pattern of behavioral recovery and residual impairment that is observed (Zigmond et al., 1990, 1993). At the lowest level of partial depletion, there are few behavioral symptoms, in part due to redundancy and in part as vacated receptors remain activated by dopamine diffusion from adjacent terminals. As the lesion becomes larger, compensation is enhanced by increased neurone firing, increased dopamine release and reduced uptake, and greater diffuse activation, but at such a level that postsynaptic activation remains sufficient to compensate for the development of most symptoms. At still higher levels of denervation, increase in dopamine synthesis and release are unable to fully compensate for the extensive loss of dopamine afferents, resulting in lasting functional impairment. Nevertheless, delayed compensation may still be possible, with the recruitment of increased TH production to enhance further dopamine synthesis in residual neurones and the development of receptor supersensitivity so that even limited pre-synaptic dopamine release has maximum post-synaptic effect.

These marked changes in sensitivity of the system not only define the asymptotic level of recovery but may also contribute to some of the marked side effects of treatment associated with advanced denervation in animals or advanced disease in man. In PD patients, as the disease (and the level of underlying degeneration) advances, so also the window of effective response to L-DOPA narrows between doses where the drug has no effect, and doses at which it induces abnormal dyskinesia but then wears off rapidly (Marsden and Parkes, 1977). Similarly in experimental animals with extensive bilateral dopamine denervation, treatment with the dopamine agonist apomorphine can substantially alleviate the profound impairment in drinking in the few days after the

lesion but thereafter, at any dose sufficient to activate the animal, the drug increasingly induces dyskinetic stereotypies which compete with the animal's ability to drink and renders the treatment ineffective (Marshall and Ungerstedt, 1976).

3.4. NEONATAL 6-OHDA AND RECOVERY OF FUNCTION

The toxin 6-OHDA does not cross the blood brain barrier after peripheral administration in the adult animal, and so must be administered centrally in order to yield effective lesions. This restriction does not apply in neonates. Thus, 6-OHDA can produce profound depletions of central catecholamines when administered subcutaneously or intracisternally to neonatal rats or mice (Breese and Traylor, 1971). Moreover, greater selectivity for individual amine pathways can be achieved by refinements of the route of delivery or by pharmacological manipulation, and different protocols of administration can allow relatively selective depletions. For example, several small repeated injections of 6-OHDA spare dopamine and preferentially deplete dopamine, whereas dopamine toxicity after a single large injection can be enhanced by pargyline treatment, in particular if the noradrenaline depletion is concurrently blocked with pargyline (Breese and Traylor, 1971; Cooper et al., 1973; Smith et al., 1973; Luthman et al., 1989).

Remarkably, rats receiving neonatal 6-OHDA lesions thrive and grow to maturity without demonstrating any major eating or drinking disorders akin to that which would be expected if lesions of similar magnitude and depletion were sustained in adulthood. Whereas the rats sustaining large dopamine depletions as neonates remained healthy, they do nevertheless show impaired physiological responses to acute homeostatic challenges (Bruno et al., 1986), along with reduced growth curves and reductions in their body size and weight (Breese and Traylor, 1971; Lytle et al., 1972; Bruno et al., 1984) in comparison to non-lesioned littermates. Similarly, at the behavioral level, whereas the neonatal 6-OHDA-treated animals do not show overt catalepsy nor impairments in locomotor activity, sensory gating or avoidance learning as would be expected if the lesions were sustained in adulthood (Cooper et al., 1973; Bruno et al., 1985; Stevens et al., 1996), they do show subtle deficits when challenged with more complex motor and cognitive tasks, such as skilled forelimb reaching, learning to escape in a spatial water maze, or acquisition and reward dependence of operant lever pressing (Whishaw et al., 1987; Archer et al., 1988; Moy, 1995; Luthman et al., 1997).

Together, these data suggest a remarkable increase of plasticity of the brain to compensate from neonatal dopamine lesions, in comparison to similar damage sustained in adulthood, an effect described in other systems of the brain as the 'Kennard' effect (Kennard, 1936; Kolb and Whishaw, 1990). Alternative explanations for this plasticity have been proposed including, changes in biochemical compensation (as described in Section 3.3 following adult lesions), and a developmental substitution of function by other systems. Support for the latter view was provided by the observation of a marked sprouting in collateral serotonergic afferents into the areas of the striatum affected by the lesion (Stachowiak et al., 1984; Berger et al., 1985; Snyder et al., 1986; Luthman et al., 1987). However, neither lesions of the serotonergic neurones (Bruno et al., 1987; Allen and Davis, 1999) nor administration of serotonin antagonists (Heffner and Seiden, 1982) block the recovery.

Rather, similar to the compensation observed with adult lesions, residual populations of dopamine neurones (which may be as little as 1 or 2%) spared by the lesion appear to underlie the remarkable compensation seen after neonatal 6-OHDA lesions also. Evidence

for this perspective is provided by the fact that the spared dopamine neurones exhibit upregulation of dopamine synthesis and turnover, and an upregulation of post-synaptic receptors (Smith et al., 1973). Furthermore, the animals exhibit reduced sensitivity to specific dopamine receptor antagonists (Bruno et al., 1985; Johnson and Bruno, 1990) but are highly sensitive to synthesis blockade by very low doses of α-methyl tyrosine (Cooper et al., 1973; Potter and Bruno, 1989; Rogers and Dunnett, 1989). Alongside evidence suggesting that there are only limited changes in most non-dopaminergic neurotransmitter systems, such as noradrenaline, acetylcholine, or adenosine (Smith et al., 1973; Herrera-Marschitz et al., 1994), and that changes that are observed in some neurotransmitter peptides are more likely to be a primary response to loss of dopamine rather than a compensatory response (Luthman et al., 1990), these data confirm the remarkable plasticity of dopamine systems to compensate for injury – even more so when sustained in early life – and emphasize the critical adaptive importance of maintaining dynamic balance in the activity of the system for the normal behavioral function of the animal.

The neonatal dopamine lesion syndrome bears some relation to (and has been proposed as a model of) a variety of human developmental and neuropsychiatric disorders, including Lesch-Nyhan syndrome and schizophrenia, in particular in the animals' changed response to agonist drugs. Thus, rats treated with 6-OHDA as neonates develop a marked sensitivity to both direct and indirect dopamine agonists. In particular, when treated with L-DOPA or apomorphine, these rats exhibit a modest locomotor hyper-activation and a range of marked stereotypical behaviors reaching their most extreme form as severe self-mutilation behavior (Breese et al., 1984a,b). A similar pattern of self mutilation is seen in the childhood developmental disorder, the Lesch-Nyhan syndrome, which is a rare X-linked genetic disorder involving disruption of the purine salvage pathway enzyme hypoxanthine-guanine phosphoribosyl transferase (HPRT; Lesch and Nyhan, 1964). Critically, the mutation is associated with a 70–90% loss of dopamine in the basal ganglia from early age (Lloyd et al., 1981; Silverstein et al. 1985). In the neonatal lesion model, the self-mutilation seen after neonatal 6-OHDA lesions appears to be mediated by supersensitivity of D1 receptors. This view is supported by evidence that the D1 agonist SCH-38393 has a greater locomotor stimulant effect in the neonatally lesioned rats, in contrast to the greater effects of D2 agonists (e.g. LY-171555) in the adult lesioned rats (Breese et al., 1985b); that self-mutilation is induced in neonatally lesioned animals (at higher doses) by SCH-38393 but not by LY-17155 (Breese et al., 1984a, 1985a); and that L-DOPA induced self-mutilation is selectively antagonized not only by broadly acting neuroleptics such as haloperidol but also by the D1 antagonist SCH-23390 (Breese et al., 1985a). The development of D1 receptor supersensitivity is dependent upon repeated priming with an agonist, and Breese and colleagues propose that in the human syndrome this could be provided by a stress component rather than pharmacologically (Criswell et al., 1989).

Interestingly, the Lesch-Nyhan model was one of the first transgenic models of a human genetic disease in mice carrying the human HPRT mutation (Kuehn et al., 1987). However the HPRT deficient transgenic mice do not exhibit detectable motor deficits or self-mutilation (Finger et al., 1988; Jinnah et al., 1992). Moreover, they do not manifest any loss of dopamine neurones, although there may be modest reductions in forebrain catecholamine levels (Dunnett et al., 1989; Jinnah et al., 1992; Jinnah et al., 1994), the magnitude of which is dependent upon age, strain and the precise region of the basal ganglia examined (Jinnah et al., 1999), suggesting the presence of an alternative cellular

purine salvage pathway to compensate in mice for the disruption of the HPRT enzyme which is so debilitating in man.

A second disorder for which the neonatal 6-OHDA lesion model has been considered relevant is schizophrenia. Thus, the neonatal 6-OHDA lesioned animals exhibit clear abnormalities in the acoustic startle response, in particular in their heightened response to dopamine agonist drugs and an inability to use a brief warning stimulus to block the startle (an effect known as 'pre-pulse inhibition', PPI; Schwarzkopf et al., 1992). A subgroup of schizophrenic patients with reduced dopamine also exhibited a similar selective impairment in PPI, leading to the suggestion that the neonatally lesioned animal may also represent a good model for this form of human psychosis (Schwarzkopf et al., 1996, 1992), in particular the relevant dependence of the deficit in sensory gating to increased sensitivity of the D1 receptor and its normalization by treatment with selective D1 antagonists (Schwarzkopf et al., 1996).

4. UNILATERAL NIGROSTRIATAL LESIONS IN RATS

4.1. STEREOTAXIC 6-OHDA (AND OTHER) LESIONS

Much of the work on biochemical plasticity at the cellular level after dopamine-depleting lesions in adult animals (see Section 3.3) has been based on making unilateral rather than bilateral 6-OHDA lesions of the nigrostriatal bundle, because it leaves one side of the brain intact and does not induce any of the gross impairments in animals' regulation of food and water intake associated with bilateral damage. The same principle applies to behavioral studies of the motor effects of dopamine depletion. Because of the cross-over (primarily at the level of the brainstem) of sensory inputs into, and motor outputs, from the brain, a unilateral lesion in the nigrostriatal pathway produces predominantly unilateral deficits on the contralateral side of the body and in responding to stimuli in contralateral space. This then allows detailed assessment of lateralized impairments, in which not only can the intact side of the brain maintain the animal in full health throughout long-running experiments (i.e. over weeks or months, as required), but also performance on the intact side of the body provides a within-animal control for deficits on the side affected by the lesion.

Lesion methods, and in particular lesion targets, can vary considerably between different studies. The most widely used lesion, introduced by Ungerstedt, is to inject the toxin stereotaxically into the ascending nigrostriatal bundle at the level of the posterior hypothalamus. At this level, the fiber bundle is at its most compact and it is the easiest to achieve the most complete lesions of forebrain projections to the neostriatum, with the mesocorticolimbic projections from the ventral tegmental area (A10) to ventral striatum, septum and other limbic targets also disrupted, although typically less completely. Conversely, studies targeted at discrete subdivisions of the projection, for example to distinguish functional differences between VTA, nigral and retrorubral projections or between different dorsal and ventral striatal targets, have adopted lesions directed at discrete cell body or striatal projection targets. By virtue of the wider distribution of cells and fiber terminals in comparison to the compact bundle of the nigrostriatal fibers themselves, such lesions are typically less complete, with correspondingly smaller deficits, and a greater tendency for transient effects prior to full recovery. However, the approach to make terminal lesions has been considerably refined in recent years, particularly with

the systematic quantitative studies of Kirik and colleagues to determine the optimal dose concentration and coordinates for the multiple deposits into striatal terminal areas that will achieve extensive focal depletions in the dorsal striatum (Kirik et al., 1998). This lesion is associated with a slowly progressive retrograde degeneration of the afferent neurones over a period of weeks (Rosenblad et al., 2000), in contrast to the very rapid degeneration of the nigrostriatal system over a period of just a few days after injection into the fiber bundle (Ungerstedt, 1971c; Hökfelt and Ungerstedt, 1973).

Although 6-OHDA has proved to be the most popular toxin for lesioning the nigrostriatal bundle in rats, other lesion strategies have also been adopted. One early approach is to physically disrupt the ascending fiber pathway by electrolytic lesion or knife cut. Previously employed in anatomical studies (Andén et al., 1964) and for pharmacological protection (Janson et al., 1988) of the nigrostriatal projection, this approach is coming back into more widespread use with the application of the Scouten knife technique (Scouten et al., 1982) to make rather precise stereotactic transaction of the fiber bundle with little surrounding damage on the approach track (Brecknell et al., 1995; Moon et al., 2000). In contrast to the rather rapid degeneration of the nigral neurones over a few days after 6-OHDA lesions of the bundle, and over 1–2 weeks after terminal 6-OHDA lesions, the degeneration is much slower after knife-cut lesions (Brecknell et al., 1995), which not only more adequately reflects the slow progressive degeneration seen in human PD, but also allows a greater time window for neuroprotection and promoting regeneration of the cells following axotomy (Wilby et al., 1999; Moon et al., 2003).

As a second alternative, following the discovery of MPTP toxicity in man, this chemical toxin has been widely used for making lesions in mice and monkeys (see Section 5.1). Although MPTP itself is not toxic in rats (due to the absence of the critical isoform of monoamine oxidase, MAO-B, to act as a substrate for its conversion), stereotaxic infusion of the active metabolite, MPP+, into the nigrostriatal pathway exhibits a similar toxicity to that achieved with 6-OHDA (Sirinathsinghji et al., 1988). However, since MPP+ requires much slower administration and is considerably more toxic to humans, there are few advantages of this strategy over the conventional use of 6-OHDA for making chemical lesions.

A wide variety of other chemicals, such as rotenone, have also been found to be toxic to dopamine neurones when administered in vitro (Sherer et al., 2003) or in vivo (Heikkila et al., 1985b; Ferrante et al., 1997; Betarbet et al., 2002). However, these studies are primarily toxicological, seeking to identify the possible causes of PD in man (Jenner, 2001), and do not replace 6-OHDA as the primary experimental tool when the need is to provide the most powerful strategy for experimental analysis of dopamine system function per se.

4.2. ROTATION

When activated, animals with unilateral dopamine lesions turn in circles, a phenomenon known as ‘rotation’ (Glick et al., 1976; Pycock, 1980; Koshikawa, 1994). The phenomenon was first described in detail by Ungerstedt (Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1971d) who at the same time proposed an automated test apparatus for recording rotation automatically – the so called ‘rotometer’ (Fig. 7; Ungerstedt and Arbuthnott, 1970; Dunnett, 1993). The availability of such a simple,

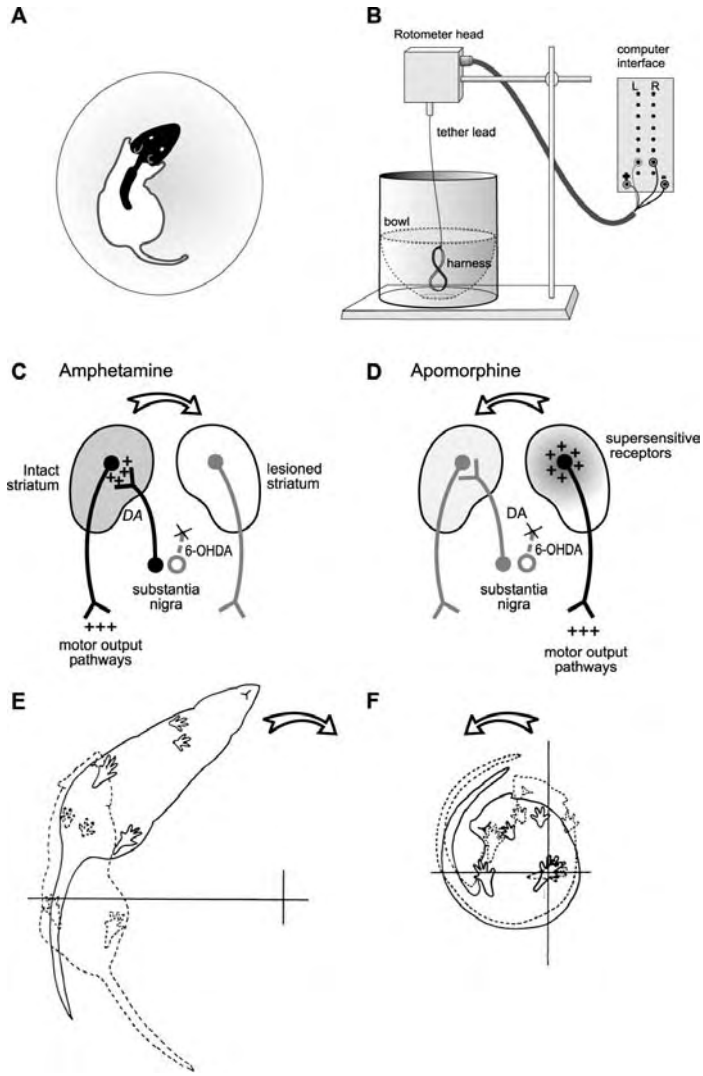

Fig. 7. Rotation after unilateral nigrostriatal lesions. **A.** Postural asymmetry in a rat that has received a recent unilateral lesion of the right nigrostriatal pathway. **B.** Illustration of automated rotometer test apparatus. **C.** Effect of amphetamine in the bilateral nigrostriatal system viewed schematically from above, acting presynaptically to induce heightened functional output from the intact striatum on the side contralateral to the lesion and producing an ipsilateral motor bias. **D.** Schematic effect of apomorphine to induce a heightened functional output on the ipsilateral side and contralateral turning bias. **E, F.** Amphetamine and apomorphine not only induce turning asymmetries in opposite directions, but there are very different morphologies to the asymmetric postures during turning to the two drugs. (Drawings of ventral views of turning rats from Koshikawa (1994) with permission.)

objectively-measurable and easily-quantifiable, behavior has made rotation one of the most widely studied phenomena in behavioral neuroscience.*

As described by Ungerstedt, following an injection of amphetamine, animals with unilateral nigrostriatal dopamine lesions exhibit a head-to-tail turning in circles at a rate of 5–15 turns per minute in a direction ipsilateral to the lesion, and lasting over a 4–5 h duration (Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1971d), similar to the locomotor activating effects of the drug in intact animals. Since amphetamine induces dopamine release and blocks its reuptake from intact nigrostriatal terminals, the turning response is most plausibly explained by a differential dopaminergic activation of motor outputs within the intact striatum in comparison to the reduced response in the striatum denervated of a dopamine input on the side of the 6-OHDA lesion (see Fig. 7C). Conversely, if treated with a receptor agonist, such as apomorphine, the animals also exhibit a strong rotational response, this time lasting for the 45–60 min of drug action, but in the opposite direction, contralateral to the lesion. On the basis of this behavioral data, Ungerstedt hypothesized that the contralateral turning could be attributable to the development of receptor supersensitivity as a compensatory response to denervation in the striatum on the side of the lesion (Fig. 7D), an effect that has subsequently been amply confirmed at the levels of upregulation of receptor binding and receptor gene expression. Nevertheless, the two types of turning should not be thought of simply as mirror images of each other since the limb stepping patterns have been described as quite distinct. Thus, apomorphine-induced rotation involves more compact tighter turns with the head down and the rat pivoting around one hind paw 'on the spot' at the base of the turning bowl, whereas by contrast the response to amphetamine involves more extended forward locomotion in a circular path, with the animal frequently rearing against the walls of the bowl or test arena (Fig. 7E,F; Ziegler and Szechtman, 1988; Koshikawa, 1994).

One of the advantages of rotation as a behavioral measure is that the rates of rotation to both amphetamine and apomorphine appear to provide good correlates of the extent of dopamine depletion induced by the lesions (Costall et al., 1976; Hefti et al., 1980; Schmidt et al., 1983; Dunnett et al., 1988), although, in our hands the correlation with amphetamine is the more reliable (Dunnett et al., 1988). In contrast to most behavioral measures which require statistical demonstration of lesion effects in groups of animals, the size of this correlation (frequently as high as $r = 0.8$ or 0.9) means that rotation can be used as a reliable non-invasive index of the effectiveness of nigrostriatal lesions in each individual animal. In experiments involving surgically complex repair strategies, such as long-term trophic factor delivery or neural grafting, this feature of rotation has allowed the experimental animals to be screened initially for ineffective lesions, and then allocated to counter-balanced treatment subgroups, making the experimental analysis of treatment effects far more reliable and powerful than can be achieved relying on random variation between animals alone (for examples, see (Björklund et al., 1980; Brundin et al., 1988; Dunnett et al., 1988)).

Rotation has been extensively studied both in terms of its pharmacology, the neural substrates, and the underlying behavioral process(es).

Pharmacology. Although lesions and drugs interacting with other neurotransmitter systems can also cause turning (Pycock, 1980), rotation effects are consistently the

*a PubMed search on 'dopamine' + 'rotation' yields about a thousand publications since Ungerstedt's original report. Ungerstedt and Arbuthnott's (1970) paper intoducing that rotometer has been citied approximately 950 times up to mid-2003.

strongest and the most reliable following pharmacological activation of dopaminergic systems in animals with unilateral dopaminergic lesions. A wide variety of pharmacological agents may be used. First, drugs which stimulate dopamine release pre-synaptically (with amphetamine as the prototype) all induce rotation in the ipsilateral direction. This includes methamphetamine, methylphenidate and phencyclidine which like amphetamine increase vesicular dopamine release; and cocaine and nomifensine which both block dopamine reuptake and hence prolong transmitter availability at the synapse (Pycock, 1980; Schwarting and Huston, 1996b). Dependence upon functional pre-synaptic terminals is demonstrated by the ability of peripheral administration of the synthesis inhibitor α-methyl tyrosine to block amphetamine induced rotation in unilateral lesioned animals (Reavill et al., 1983), and the observation that amphetamine will induce rotation in unlesioned animals following local blockade of striatal dopamine synthesis unilaterally by injection of α-methyl tyrosine into the striatum on just one side (White and Tapp, 1977).

Secondly, dopamine agonists typically induce contralateral turning by action at receptors, in particular of the D2 class, rendered supersensitive as a result of the lesion (Pycock, 1980; Schwarting and Huston, 1996b). The fact that partial forebrain depletions after small lesions typically compensate by presynaptic adaptation (see Section 3.3) and receptor sensitivity only changes after much larger lesions, results in the fact that apomorphine rotation typically requires more extensive lesions before turning becomes apparent. However, at the highest levels of dopamine cell loss, the rate of turning in the contralateral direction to low doses of agonist can be as rapid as that seen in the ipsilateral direction after amphetamine (Hefti et al., 1980; Dunnett et al., 1988). Apomorphine is used very widely as the prototypical agonist, acting at both D1 and D2 receptors, but probes with selective receptor agonists indicate that both classes of receptor are implicated in the turning response. Thus, whereas selective D1 or D2 agonists alone can induce weak contralateral rotation, mixed agonists have greater effects than selective agonists, and the co-administration of D1 and D2 agonists produces greater rotation to that seen after either alone (Miller and Beninger, 1991; Schwarting and Huston, 1996a). However, the influence of the two classes of receptor on rotation do have different properties: rotation to D1 agonists such as SKF-38393 takes approximately 2–3 weeks after lesion to develop and is promoted by priming, whereas rotation to D2 agonists, such as quinpirole or pergolide appears much more rapidly after lesion and is affected less by priming (Schwarting and Huston, 1996a).

L-DOPA induces contralateral rotation in unilaterally lesioned rats, similar to receptor agonists, although by a mechanism that remains unclear (Ungerstedt, 1971b; Schwarting and Huston, 1996a). L-DOPA is converted to dopamine by the enzyme dopa-decarboxylase, and this is believed to take place predominantly in pre-synaptic terminals. However, the fact that L-DOPA-induced rotation is not only dependent upon the upregulation of post-synaptic D2 receptor binding but is also at its strongest in the most denervated cases (Heikkila et al., 1981; Thomas et al., 1994) suggests that the conversion of L-DOPA to dopamine need not take place exclusively in spared dopaminergic terminals; serotonergic terminals and glia may also contribute (Schwarting and Huston, 1996a).

A two-process model. As originally characterized by Ungerstedt, rotation after unilateral nigrostriatal lesion was originally considered as reflecting a net asymmetry in dopaminergic activation of the striatum in the two hemispheres (Andén et al., 1966; Ungerstedt 1971b,d). However, subsequent studies indicate that rotation is dependent

upon a convergence of two distinct functional processes: a behavioral asymmetry mediated by the dorsal neostriatum combined with locomotor activation mediated at the level of the ventral extension of the striatum in the nucleus accumbens (Kelly, 1977; Pycock and Marsden, 1978). The dual process is most apparent in studies which combined selective lesions. Thus, animals with a unilateral 6-OHDA lesion in the dorsal striatum alone rotate ipsilaterally in response to amphetamine, but fail (in the absence of supersensitive receptors in the ventral striatum) to rotate in response to apomorphine. Conversely, other animals with bilateral 6-OHDA accumbens lesions in addition to the unilateral dorsal lesion do rotate contralaterally to apomorphine, but no longer (in the absence of an intact innervation of the ventral striatum on either side) rotate to amphetamine (Kelly and Moore, 1976, 1977; Pycock and Marsden, 1978). The role of the ventral striatum bilaterally in locomotor activation per se has already been discussed (Section 2.2). The motor asymmetries and side biases associated with the dorsal striatum are apparent in a variety of other experimental manipulations also. Thus, electrical stimulation of the striatum also induces head turning and contralateral responding, effects blocked by peripheral injections of antagonists or lesions in output systems (Zimmerberg and Glick, 1974; Barnett and Goldstein, 1975), and injection of dopamine or a dopamine agonist into the dorsal striatum induces contralateral head movements and modest turning (Ungerstedt et al., 1969; Costall and Naylor, 1974;), but not full rotation.

Spontaneous rotation. It is not only with lesions that rats will exhibit circling. Glick and colleagues have shown that if a large group of intact rats is given amphetamine or apomorphine peripherally, a proportion of them will exhibit reliable turning predominantly in one direction as a component of their locomotor activation, and this can be recorded by testing normal animals under the drug in rotometer bowls (Jerussi and Glick, 1974, 1975). The amphetamine but not the apomorphine response is blocked by α-methyl tyrosine, whereas both responses are blocked by haloperidol (Jerussi and Glick, 1975, 1976). Of particular interest is the fact that although the magnitude and direction of turning differs between rats, these factors are consistent for each rat and are reflected by an increased release of dopamine in the striatum on the side contralateral to the direction of spontaneous turning (Glick et al., 1974; Jerussi and Glick 1976). Intriguingly, a spontaneous mutation has recently been described in 'circling' rats with a spontaneous tendency to rotate, and again the direction of circling reflects higher levels of dopamine in the striatum on the contralateral side (Richter et al., 1999; see Section 6.1).

Conditioned rotation. A related phenomenon reflecting motor asymmetries associated with changes in striatal dopamine release is the demonstration that intact animals can be trained to turn in circles in just one direction as an operant conditioned response for water reward (Yamamoto and Freed, 1982; Dunnett, 1993). Trained rats exhibit an increase in striatal dopamine and DOPAC within the intact neostriatum on the side contralateral to the direction of turning, measured both postmortem (Yamamoto and Freed, 1982, 1984) and in vivo (Yamamoto et al., 1982). As increased turnover was associated with an increased TH activity (Morgan et al., 1984) leading to an increase in the dopamine synthesis (Bennett and Freed, 1986). Conversely, the animals' ability to acquire the turning response is blocked by lesions in the contralateral nigrostriatal pathway, facilitated by lesions in the ipsilateral pathway (Dunnett and Björklund, 1983; Richards et al., 1990) and restored by nigral grafts (Dunnett et al., 1986). However, there has been some disagreement about whether the effects of conditioned motor responding are laterally specific, since others have reported bilateral rather than unilateral changes following conditioning (Szostak et al., 1986, 1989; Schwarting and Huston, 1987; Glick and Carlson,

1989). Moreover, animals forced to turn in circles by running on a circular treadmill, as opposed to those trained to turn laterally as a conditioned response, also show bilateral rather than unilateral elevations in dopamine turnover (Sabol et al., 1990).

Motor asymmetries associated with conditioning of dopaminergic activation have been observed in another context. Carey showed that if animals with unilateral lesion are consistently tested for drug-induced rotation in one environment, and given control injections of saline (which do not induce rotation) in a second environment, then the turning response itself becomes conditioned to the environment so that subsequent injection of saline in the first, trained environment will now induce turning in the direction associated with the previous drug treatment (Carey, 1986). Thus, if previously treated with apomorphine, the animals would exhibit contralateral rotation in that environment when injected with saline, whereas if the previous conditioning had been with amphetamine they would rotate ipsilaterally to the same saline probe (Carey, 1986; Dunnett et al., 1986). Whereas both drug-induced rotation response and spontaneous ipsilateral turning in a novel environment are dependent upon dopamine receptor-mediated mechanisms, as demonstrated by blockade with selective D1 and D2 antagonists, conditioned turning is not affected by these drugs and so appears to be mediated primarily by non-dopaminergic mechanisms rather than providing an index of dopaminergic asymmetries at the time of testing (Carey, 1990).

4.3. SIMPLE MOTOR AND SENSORIMOTOR TESTS

Rotation provides a very simple measure of the asymmetry in motor activation associated with dopamine lesions. However, PD patients have a more complex range of motor deficits, not just in akinesia and rigidity, but in the initiation of voluntary and purposive movement, and in fine motor control. To develop the validity of the animal models, we need to determine the extent to which dopamine denervation in experimental animals produces comparable impairments in simple motor behaviors not dependent on pharmacological activation, and in more complex behaviors whilst controlling for confounding effects of simple performance deficits.

The simplest measures of motor performance are to be found in asymmetries of posture, gait and balance. Unilateral 6-OHDA lesioned animals can exhibit marked postural deficits on the ipsilateral side and turning of the head to the ipsilateral side (Marshall et al., 1974; Henderson et al., 2003). Even though these tend to recover rapidly over the first few days after the lesion, the side bias can readily be re-elicited in the form of rotation following administration of an activating drug (Section 4.2). Animals will also show turning to the side of the lesion when stressed. Thus, pinching an animal's tail, which is activating for a normal rat, will induce robust turning for the duration of the stimulus in rats with unilateral lesions (Chiodo et al., 1979). More simply, the animals can simply be lifted by the tail and will exhibit a postural twisting of the body about the longitudinal axis towards the side of the lesion (Borlongan and Sanberg, 1995; Henderson et al., 2003).

An animal's gait while walking can be simply measured by applying paint or dye to the paws and analyzing the track of the footprints (Schallert et al., 1978). The coordination and the sequencing of paw movements in footprint tests of the coordinated gait are severely disrupted after intraventricular 6-OHDA lesions (Pellis et al., 1987), but the effects of unilateral lesions have not so far (to the author's knowledge) been reported. Nevertheless rats with unilateral nigrostriatal lesions are severely impaired in coordination

and balance on a raised beam, on which they exhibit more slips of the fore- and hind-paws contralateral to lesion (Schallert et al., 2002) or falls from a slowly rotating rod, in which the magnitude of the deficit is proportional to the extent of depletion as determined by amphetamine-induced rotation testing (Rozas and Garcia, 1997; Rozas et al., 1997; Makanjuola et al., 2003).

Other aspects of the animals' impairment in forelimb placement and movement are seen in specific aspects of forepaw placing. Thus, in the cylinder test, a rat is placed into a glass cylinder, in which it rears by placing the two forepaws against the cylinder side to balance, and using the paws to initiate sideways stepping movements. Rats with unilateral 6-OHDA lesions show reduced numbers of placements with the contralateral paw and never initiated a sequence of stepping movements with that paw (Schallert and Lindner, 1990; Schallert and Tillerson, 1999). Similarly, in a forced stepping test, rats are loosely held by the body and allowed to place one or the other forepaw on the bench surface. When a normal rat is moved sideways, whether toward or away from the side of the placing paw, the rat makes a series of corrective stepping movements to keep the paw in contact with the table (Schallert et al., 1979). Corrective stepping is disrupted by the paw contralateral to a unilateral lesion (Olsson et al., 1995; Kirik et al., 1998; Rosenblad et al., 1998; Schallert et al., 2000).

Although these tests of specific stepping and placing movements may be considered to provide an analysis of the animals' motor deficits, they also clearly relate to the sensorimotor impairments, first described by Marshall, after lateral hypothalamic lesions. Having developed a battery of tests to evaluate 'sensory neglect,' animals with unilateral nigrostriatal lesions were seen to exhibit similar deficits in particular on the contralateral side of the body. Thus, the animals failed to orient to lateralized visual, olfactory and tactile stimuli applied to the body surface (Marshall et al., 1974), the deficits can be alleviated acutely by apomorphine during the first few days after lesion (Marshall, 1979), and the rats exhibit a slower spontaneous recovery progressing in a rostro-caudal gradation over the subsequent month (Marshall, 1979). Marshall combined his measures of neglect to explicit stimuli with other measures of motor response to stimuli, such as righting responses, turning on inclined grids, and forepaw placing reactions when the animal's snout approaches and the whiskers contact a table surface or corner (Marshall et al., 1974; Schallert et al., 2000). One of the major difficulties in using the original Marshall battery is that it primarily involves the observer ratings of the rats' performances. These can provide a powerful guide to description of the neurological impairment but are subjective and difficult to quantify. We and others have attempted to standardize the scoring systems, which has proved useful for evaluating the effects of different lesions and of reparative treatments, such as transplants (Björklund et al., 1980; Dunnett and Iversen, 1982). Other investigators have added a variety of additional tests of sensory neglect. For example, in the 'sticky label' test, small pieces of the adhesive tape are applied around the wrists of the two forepaws. While the intact rats rapidly remove both the labels, rats with unilateral 6-OHDA lesions are slow to contact and remove the label on the contralateral paw (Schallert et al., 1982, 1983, 2000), a deficit that can be alleviated by ipsilateral lesions of the subthalamic nucleus (Phillips et al., 1998). Similarly in the 'disengage' test, rats are distracted with a laterally applied probe while eating a piece of chocolate. A normal rat will rapidly orient and respond to the distraction, but a lesioned rat neglects the stimulus on the side contralateral to the lesion, even when it has fully recovered normal responding to primary tactile stimuli when undistracted (Schallert et al., 1982; Schallert and Hall, 1988; Mandel et al., 1990). In contrast to the sticky labels,

6-OHDA lesion deficits in contralateral performance on this test are not alleviated by STN lesions (Henderson et al., 1999).

Marshall argued that the deficits elaborated in his neurological tests were neither purely sensory, because the animals would flinch but not orient to tactile stimulation, nor purely motor, because involuntary reflexes, such as the vestibular-driven righting response remained intact, rapid and accurate. Rather, the lateral hypothalamic and by extension the nigrostriatal deficit is best characterized as a 'sensorimotor' impairment in generating coordinated responses to lateralized stimuli. In an early attempt to determine whether these deficits are attributable to sensory, motor or integrated functions, Marshall, Turner and Teitelbaum (1971) trained animals to make either ipsilateral or contralateral head turns to escape a lateralized electric shock applied to one hind paw. Unilateral lesions did not disrupt making an ipsilateral response to a contralateral stimulus (so detection of the stimulus per se was not disrupted) nor making a contralateral response to an ipsilateral stimulus (so motor execution of the lateralized escape response was not the primary problem). Rather, the animals were only impaired when both stimulus and response were applied to the contralateral side, corroborating the initial hypothesis that the deficit is 'sensorimotor' in nature. Unfortunately, the lateralized S-R escape task used here was complex, and was applied only to lateral hypothalamic and amygdala, but not the nigrostriatal, lesions. However, this experiment introduces an important conceptual strategy for investigating the laterality of sensory and motor impairments, which (as outlined in Section 4.4) has yielded somewhat different conclusions, when applied to the nigrostriatal dopamine system.

4.4. SKILLED MOTOR CONTROL

Skilled motor control has been evaluated in tests of manipulative ability, such as a paw reaching for food, in tests of lever pressing, and in simple and choice reaction time tasks in operant test apparatuses.

Skilled paw reaching. A variety of strategies have been employed to assess handedness and reaching skills of rats. Typically, food-deprived animals are allowed to reach and retrieve pieces of food through a slot in the cage floor or wall (Castro, 1972; Whishaw et al., 1997a), into tubes (Siegfried and Bures, 1980; Pisa, 1988), from a tray (Whishaw et al., 1986), from the steps of a staircase (Montoya et al., 1990; Abrous and Dunnett, 1994), or from a moving conveyor belt (Evenden and Robbins, 1984). Many of these tests allow graded levels of difficulty by varying the depth of the tube (Siegfried and Bures, 1980), the width of the gap between the cage and the tray (Whishaw et al., 1986), the speed of the conveyor (Evenden and Robbins, 1984), or the different steps of the staircase (Montoya et al., 1990; Abrous and Dunnett, 1994). Equally, several of the tests have sought to construct the apparatus so that the rat is forced to use just one or just the other paw in different configurations, so as to be able to assess the changes in reaching skills with the two limbs independently, following unilateral lesions. Normal rats use either forepaw to retrieve pellets. However, following unilateral 6-OHDA lesions, most rats will by choice use only the ipsilateral paw (Siegfried and Bures, 1980; Evenden and Robbins, 1984; Whishaw et al., 1986; Dunnett et al., 1987). Residual contralateral performance can nevertheless be probed by restricting the use of the ipsilateral limb using a cuff (Whishaw et al., 1986) or an apparatus in which the staircase or tube is positioned so that the animal can only physically use the contralateral limb (Montoya et al., 1990; Abrous et al., 1992). When this is done, reaching performance falls dramatically (Whishaw et al., 1986;

Montoya et al., 1990; Abrous et al., 1992). Lesions restricted to the discrete areas of the striatum, suggest that dopaminergic innervation of more lateral areas is likely to be critical for skilled-reaching performance (Sabol et al., 1985; Pisa, 1988; Pisa and Cyr, 1990).

A detailed analysis of the reaching movements indicates that the lesioned animals are largely unable to make independent reaching movements with the contralateral limb (Whishaw et al., 1986). When reaching through the bars of a cage to retrieve pellets from a tray, if the animals did fortuitously manage to grasp a pellet with the affected limb, rather than turn the paw as normal to place the food into the mouth, they would rotate their heads underneath the paw to try to bite the pellet (Whishaw et al., 1986). In a related test in which the rats simply needed to pick up the pieces of food, the affected paw was impaired in picking up and grasping the food, grasp was only with a whole paw grip and did not permit manipulatory movements, and the animals did not open the paw to release their grip in taking the food into the mouth or to regain support, once the food was eaten (Whishaw et al., 1997a). In the staircase test, the rats showed severe impairments not only in reaching success with the contralateral paw, but also in a reduction in the numbers of reaching attempts (Whishaw et al., 1997b). Control reaching is a well coordinated sequence in which the paw is lifted as the first stage in aiming at a particular target, pronated and the digits opened as the paw approaches the pellet, the food is grasped by flexion of the digits with the paw stationary, and then the paw is supinated to face medially as the limb is withdrawn, so that as it reaches its mouth the rat can take the food easily from the paw with the mouth. By contrast, when using the limb contralateral to a unilateral 6-OHDA lesion, the paw slips off the shelf rather than being lifted and aimed, the rats reach towards the pellet without pronation or opening of the digits, they clasp the pellet against the side of the platform by abduction of the paw and scoop it upwards towards the snout, from where it is retrieved by the mouth (Whishaw et al., 1997b). Thus, Whishaw and colleagues have argued that the rats' difficulty seems to involve an inability to control a precise step-by-step motor sequence, not just a failure to initiate an established motor routine. A number of studies have sought to alleviate this impairment with different treatment strategies, so far without great success (Dunnett et al., 1987; Montoya et al., 1990; Abrous et al., 1993; Olsson et al., 1995; Emgård-Mattson et al., 1997; Kirik et al., 2001).

Lever pressing tests. Further analysis of the paw-reaching response has been sought by analysis of pressure and latencies of responding in operant lever pressing apparatus. For example, after training rats to lever press for food pellet reward on a continuous reinforcement schedule and recording the preferred paw use, rats received contralateral 6-OHDA lesions, which produced a marked switch in the preference to using the ipsilateral, unaffected paw in subsequent tests in the operant chamber (Uguru-Okorie and Arbuthnott, 1981). After training, normal rats can be readily trained to switch to use of the nonpreferred paw, either by differential reinforcement only when they used the nonpreferred paw or by injection of the local anesthetic lignocaine into the flexor muscles of the preferred paw. However, following a unilateral nigrostriatal 6-OHDA lesion, none of the rats were able to either continue, or to learn anew, using the affected paw (contralateral to the lesion) for pressing the lever, whether trained by differential reinforcement or by immobilization of the unaffected paw (Hamilton et al., 1985).

Analysis of the regional changes in dopamine turnover in rats trained to use just one forelimb for bar pressing indicates significant increases in dopamine turnover bilaterally, in particular in the posterior and the lateral areas of the striatum (Church et al., 1986). In like vein, Cousins and Salamone trained rats on a fixed ratio 5 (FR5) schedule of

reinforcement, which allows measurement of the initiation and duration of individual responses in the runs before each reinforcement. Dopamine turnover was seen to increase in the ventrolateral striatum of normal animals during performance of the FR schedule (Cousins and Salamone, 1996b). Conversely, dopamine depletion of the ventrolateral striatum by direct injection of 6-OHDA into the terminal areas bilaterally produced a marked depletion in the number of lever presses per session and a marked increase in the response initiation times, an increase in the incidence of long pauses, but only a marginal effect on response durations (Cousins and Salamone, 1996a,b). Injections of L-DOPA have been seen to partially reverse the deficit in FR5 responding (Cousins and Salamone, 1996a).

There is, however, a difficulty in interpreting the outcome of these tests of free operant performance, in that it is extremely difficult to distinguish whether the recorded impairments involve an underlying deficit in the motor, sensory or motivational function (Hamilton et al., 1985; Cousins and Salamone, 1996b). Attention has therefore turned to tests in which animals are trained to make rapid movements in response to an imperative stimulus in discrete trial tasks, in which specific changes in signal detection, choice accuracy, reaction time and movement latency can potentially distinguish sensory, motor and motivational components of the deficit.

Simple reaction time. Spirduso and colleagues introduced a simple reaction time paradigm for rats in which the animals are trained to hold down a response lever until a stimulus (light or tone) signals releasing the lever as rapidly as possible to escape and avoid shock. Rats would rapidly learn this lever release avoidance response with a reaction time in the 100–300 ms range (Spirduso et al., 1981), similar to that recorded in human reaction time tasks that are disrupted in PD. Variations between animals in the speed of movement is predicted by the levels of dopamine-receptor binding in the striatum (Spirduso et al., 1984), and both successful avoidance and reaction times are impaired by dopamine antagonists, such as chlorpromazine, SCH-23390 and spiperone (Spirduso et al., 1981; Mayfield et al., 1993), or by intrastriatal injections of 6-OHDA (Spirduso et al., 1985).

Although avoidance of foot shock as used in these original studies provides for very rapid training and fast responding, welfare considerations have led most subsequent studies to utilize positive reinforcement, with the animals trained to release the lever rapidly to the imperative stimulus for food reward. Many labs have now replicated the utility of the lever release paradigm as a sensitive measure of dopaminergic and basal ganglia involvement in the initiation of learned S-R responses (or motor 'habits'; Amalric and Koob, 1987; Brenner and Mirmiran, 1988; Marrow et al., 1993; Florio et al., 1999; Hauber et al., 2001; Gulley and Rebec, 2003). Thus, for example, in one study, neuroleptics and selective D1 and D2 antagonists all disrupted the numbers of trials completed, whereas chlorpromazine but not haloperidol, and the D2 antagonist raclopride but not the D1 antagonist SCH23390 significantly slowed reaction times (Marrow et al., 1993). Amalric and colleagues in particular have provided extensive characterization of the basal ganglia systems affecting lever release responding. Thus, reaction times are slowed by peripheral injections of the dopamine antagonist flupenthixol i.p. but, since this also reduced the numbers of trials initiated by the rats, the specificity of the effect is hard to determine (Amalric and Koob, 1987). However, reaction times were also lengthened by bilateral 6-OHDA lesions in the posterior neostriatum, whereas the numbers of trials were unchanged in this case, indicating that the deficit involves a specific slowing of initiation of conditioned goal-directed motor responses to sensory cues, rather than being attributable

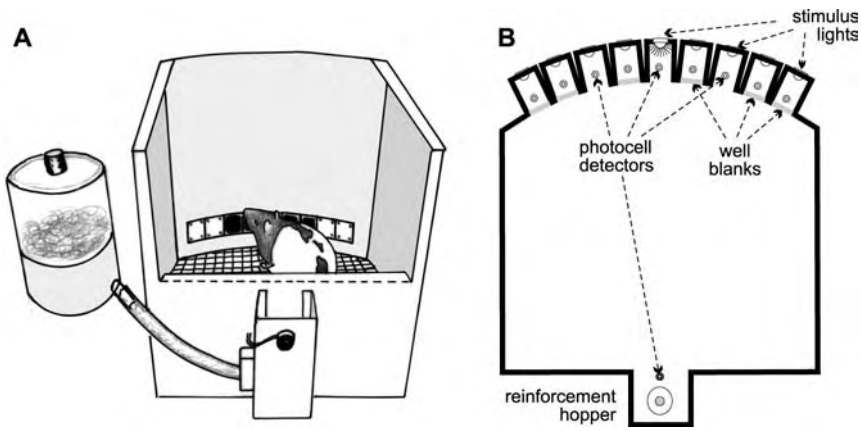

Fig. 8. Nine-hole box test apparatus for assessing serial and choice reaction time. **A.** Perspective view. **B.** Schematic plan view.

to general motivational changes. In the same study, lesions of the nucleus accumbens had no effect. The magnitude and specificity of the conditioned reaction time deficit depends on both the locus and the magnitude of the depletion. Thus, whether the lesions are made in the nigrostriatal bundle (Smith et al., 2002) or in the terminal areas of the striatum (Amalric et al., 1995b), small lesions have little effect, moderate depletions focused on dorsal lateral parts of the striatum provide selective deficits in slowing of reaction time, whereas the large and more widespread lesions produce a more general disruption of all aspects of performance. Moreover, the deficit can be alleviated by compensatory lesions in the indirect output pathway in the STN (Amalric et al., 1995a; Baunez et al., 1995). However the STN lesions also increase premature responding in their own right (Baunez et al., 1995), confounding the interpretation of whether this is a specific reversal of the deficit as opposed to independent and additive effects of the two lesions. More clear-cut evidence of a specific recovery after 6-OHDA lesions has been reported in rats receiving dopamine-rich nigral grafts, demonstrated by a speeding of reaction times and more rapid recovery to preoperative levels of performance (Moukhles et al., 1994), suggesting that restoration of functional dopaminergic transmission is required to promote good recovery in skilled motor tasks of this type.

Choice reaction time. A related operant test of reaction time performance in rats, but in a very different apparatus, is provided by the studies initiated by Robbins and his colleagues in the 'nine-hole box.' In this apparatus (see Fig. 8), one wall of the test box provides a curved horizontal array of nine response holes, each of which contains a photocell sensor to detect a nose poke response and a light that can be illuminated to provide specific stimuli; a food magazine is positioned in the middle of the opposite wall, and house lights are used to provide general illumination as well as to signal intertrial intervals and errors by 'time out' (Robbins et al., 1993). In the 'Carli' task, all but three holes are blanked off, the center hole is lit, and the rat is trained to hold its nose in the center hole for a variable duration (0.2–1.0 s) until one of the two side holes is illuminated. In the 'Same' version of the task the rat is given food reward for responding to the lit side hole and punished with time out for responding to the unlit side hole; in the Opposite task the converse contingencies apply (Carli et al., 1985; Robbins et al., 1993). In the initial study in this task, separate groups of rats were trained on either the Same or

Opposite contingencies, and then received unilateral 6-OHDA lesions in the neostriatum. Following the design logic of Turner ((1973); Section 4.2) to discriminate sensory neglect from motor deficits after unilateral lesions, the rats of both groups were impaired in responding to the side contralateral to the lesions, irrespective of the side of the stimulus (contralateral in the Same group but ipsilateral in the Opposite group). Four different measures of performance were collected: in both versions of the task, the lesioned animals showed a response bias ipsilateral to the ipsilateral side; response accuracy was severely disrupted on trials where the stimulus signaled a correct contralateral response; the animals were markedly slowed in 'reaction time' to withdraw their nose from the central hole in response to a stimulus signaling a contralateral response; but showed no impairment in the 'movement time' between the withdrawing from the central hole and the nose poking the signaled hole on either side (Carli et al., 1985). This pattern of deficit is interesting because it was the 'reaction time' component (involving a *nonlateralized* withdrawal of the nose from the central hole) that was disrupted, whereas the time taken to make the lateralized movement was unaffected. This clearly suggests that striatal dopamine is necessary for the efficient *initiation* of a trained action (or motor 'habit') rather than in the *execution* of the movement per se (Carli et al., 1985). In a subsequent study, this specific profile of impairment in initiating contralateral responses was replicated with dorsal striatal lesions, whereas lesion in the nucleus accumbens did not impair performance (Carli et al., 1989). Moreover, although deficits associated with terminal lesions do eventually recover (see Section 3.3), the full impairment can be re-precipitated with low doses of α-methyl tyrosine (Carli et al., 1989).

Analysis of the contralateral deficit has been expanded by development of variations of the choice reaction time task to probe specific aspects of performance. Brown introduced a variant in which rats were trained to detect and respond to different locations on the same side of the body, and found that striatal 6-OHDA lesions disrupted choice responding only on the contralateral side, inducing a profound bias to respond to the nearer of the two holes and a slowing of reaction times when responses were correct (Brown and Robbins, 1989). Probe trials with neither or both stimuli presented suggested that the deficit was not due to a failure to detect or localize the stimuli but an impairment in the ability to direct responses in contralateral space (Brown and Robbins, 1989). In another variation, rather than requiring rats to detect spatially lateralized stimuli, Phillips and Brown (1999) trained them to make a visual discrimination – bright stimuli-go left, dim stimuli-go right (or vice versa in half the rats). Again, unilateral dopamine lesions induced ipsilateral bias, more errors and a slowing of the reaction time on the contralateral side, and this deficit was significantly alleviated by STN lesions (Phillips and Brown, 1999). In a third variation, the benefit that is seen in simple over choice reaction time tasks, provided by advance knowledge of the required response, was probed. Even though, as the earlier reaction times on the contralateral response trials was slowed by unilateral 6-OHDA lesions, the faster reaction times on the simple, over the choice, trials was maintained (Brown and Robbins, 1991). Similarly, when attention toward or away from the side of the imperative stimulus is manipulated, although unilateral lesions impair both the speed and accuracy of responding on the contralateral side, reaction times did not change differentially depending on whether the rats were required to maintain, disengage or shift their attention to the side of the stimulus (Ward and Brown, 1996). These data all converge on corroboration for the hypothesis that lateralized deficits induced by unilateral dopamine depletions, manifested as a neglect of contralateral stimuli and a slowing of the response to them,

are attributable to motor rather than attentional impairments. More specifically, the impairments seem to involve in particular an 'intentional' impairment in the selection and initiation of appropriate actions in contralateral space, rather than in the actual execution of the movement with the contralateral musculature (Hauber, 1998).

Florio and colleagues have used a paradigm that combines features of the lever release simple reaction time and the choice reaction times of the Carli task, in that they are trained in a two-lever box to hold down one lever until a light stimulus signals release of the first lever (reaction time) and the requirement to press the second lever (movement time) for food reward. The rats were always trained on the same sequence before receiving serial bilateral lesions, first on the side contralateral to the trained movement, retesting, then on the ipsilateral side, and retesting again (Florio et al., 1999). The lesions placed more ventral in the neostriatum induced more error responses than did dorsal lesions, and both reaction times and movement times were slowed, but only after the lesions had been sustained bilaterally. Thus, the impairments reflect some aspects of the specificity reported by Carli (Carli et al., 1985, 1989), but the deficit was more modest, associated with somewhat smaller and more focal depletions in this study.

The 9-hole box apparatus has been used with different schedules to evaluate the role of the dopamine systems in tests of sustained and divided attention, in which the rats have to detect and respond to brief light stimuli in any one of five open holes (Baunez and Robbins, 1999). Although this experiment was primarily designed to consider the effects of STN lesions, which produce a general disruption of performance in the attentional task (Baunez and Robbins, 1997), 6-OHDA lesions of the dorsal striatum had no effect on accuracy but lengthened latencies of responding correctly and increased the numbers of omissions and perseverative errors (Baunez and Robbins, 1999).

5. OTHER TOXIN MODELS

5.1. MPTP IN MAN, MONKEY AND MOUSE

MPTP toxicity in man. The toxin 1-methyl-4-phenyl-tetrahydropyridine (MPTP) was discovered to induce selective degeneration in forebrain dopamine systems by accident, with an appearance of a PD-like syndrome in a group of US drug addicts following self-administration of a heroin-analog designer drug. On analysis the key contaminant was found to be MPTP as a side product of a poorly-conducted synthesis of the target drug meperidine (Davis et al., 1979; Langston et al., 1983). The syndrome in affected cases presented as moderate to advanced PD of rapid onset in young people, with all the cardinal symptoms of rigidity, bradykinesia, tremor and impairment of gait and facial expression. Moreover, the MPTP syndrome is responsive to the L-DOPA treatment, just like idiopathic PD. Critically however, in the most severely affected patients, the effectiveness of L-DOPA rapidly wore off and the problems associated with long-term treatment in idiopathic disease – shortening duration of action, narrowing of the effective dose window, 'on-off' fluctuations in the drug response, and the development of peak-dose dyskinesias – all developed much more rapidly in the MPTP patients (Langston and Ballard, 1984), who still remain profoundly Parkinsonian, now, thirty years later. Imaging investigation by positron emission tomography (PET) indicates a profound loss of fluorodopa binding in the caudate nucleus and putamen in the affected patients. A lesser reduction in binding has been reported in other addicts, who had taken the same

contaminated batch of drug, but did not develop clinical symptoms (Calne et al., 1985), and there is considerable interest in determining whether this, in conjunction with natural age-related decline in normal dopamine levels of the brain, may impose an increased risk of their developing the disease in later life. Direct comparison between behavioral and biochemical changes in idiopathic and MPTP-induced PD indicated very similar symptoms and changes in dopamine and its metabolites (Burns et al., 1985). However, idiopathic disease affects all monoamine systems, whereas the noradrenaline and serotonin neurones are considerably less affected (if at all) than dopamine in the toxin-induced disease (Burns et al., 1985; Feldman et al., 1997).

The discovery of MPTP as a potential toxin has precipitated three major lines of research: first into the development of more valid functional animal models of human PD, which has been undertaken in particular in primates; second, into the mechanisms of toxicity, studied particularly in mice; and third, into the development of novel therapeutics from each of these first two lines of animal research, that are feeding back into developing clinical practice.

MPTP toxicity in primates. Following peripheral administration, MPTP induces a similar Parkinsonian syndrome in monkeys, and pathological examination reveals a profound loss of neurones in the substantia nigra, the appearance of degenerating dopamine terminals in the striatum and a loss of dopamine and HVA from the striatum (Burns et al., 1983; Forno et al., 1984; Langston et al., 1984). By contrast, locus coeruleus, nucleus accumbens and olfactory tubercle were less depleted in the early studies, but have been found to be affected, along with projections to frontal cortex as well as the striatum, in other reports dependent upon different parameters for administration of the toxin (Elsworth et al., 1990; Hornykiewicz and Pifl, 1994).

One of the difficulties with the MPTP experimental model is that there can be considerable differences in response, not only from study to study but also from animal to animal. Typically, clear pathology requires multiple doses over a period of time, and the stability of degeneration with long-lasting impairment is critically dependent on chronic administration (Feldman et al., 1997). Thus, with a single large dose, the animals can show a profound toxic reaction, and the classical syndrome associated with bilateral 6-OHDA or lateral hypothalamic lesions involving akinesia, aphagia and adipsia is readily precipitated after several doses. However, initially severely-affected MPTP primates, like rats with other bilateral lesions, can quickly recover from just one or two doses. A chronic administration paradigm is required to achieve stable lasting deficits that can be used as an experimental model of the human disease. In general, repeated small doses at high frequency over a long duration yield the most stable and consistent functional impairment (Bédard et al., 1992; Hantraye et al., 1993; Varastet et al., 1994). Moreover, whereas the early studies using a few large doses typically induced a regionally nonselective lesion of dopamine neurons, a more gradual administration regime causes preferential depletion in the putamen vs. the caudate nucleus (Moratalla et al., 1992), similar to the topography seen in the human disease. The most effective depletions for the experimental pharmacological studies may best be achieved by titrating administration of the toxin, with decisions of the dose and timing of injections based on objectively defined criteria determined from the pattern of expression (and recovery) of motor symptoms.

Once a stable chronic model has been established,the affected primates exhibit a typical Parkinsonian syndrome, for which a variety of behavioral measures have been developed. These include neurological descriptions of symptoms typical of human PD including action and resting tremor, cogwheel rigidity, postural impairments, hypokinesia and

bradykinesia (Hantraye et al., 1993; Smith et al., 1993; Gerlach and Riederer, 1996; Emborg et al., 2003). The monkeys exhibited the blank staring faces and reduction of the blinking characteristic of the human disease (Hantraye et al., 1993; Taylor et al., 1999). A variety of rating scales have developed to rate Parkinsonian signs in the MPTP-treated monkeys (Kurlan et al., 1991a; Gomez-Mancilla and Bedard, 1993; Smith et al., 1993; Benazzouz et al., 1995; Papa and Chase, 1996; Schneider et al., 1998) akin to the Unified Parkinson's Disease Rating Scale (UPDRS) used for PD patients (Fahn et al., 1987). Although large monkeys, such as rhesus and baboons, typically do develop tremor as one of their key Parkinsonian signs (Burns et al., 1983; Schultz et al., 1985; Degryse and Colpaert, 1986; Hantraye et al., 1993; Emborg et al., 2003), early reports suggested that the smaller New World species, such as marmosets and squirrel monkeys were less susceptible to tremor (Jenner et al., 1984; Langston et al., 1984; Temlett et al., 1988). However, tremor can also be seen in these species under appropriate dosing regimens (Costa et al., 2001). Comparison between the primate rating scales indicates moderate, but not full comparability (Imbert et al., 2000). Taylor and colleagues have therefore subjected different measures from rating scales, alongside formal neurological tests, to factor analysis in order to generate a combined numerical 'Parkinsonian summary score' for primates (Taylor et al., 1994, 1995). In addition, many investigators have used automated measures of decreases in the home cage locomotor activity and other behaviors to quantify the magnitude of deficit (Pearce et al., 1995; Chassain et al., 2003). Thirdly, deficits on more formal tests of skilled movement, and problem solving in tests with both motor and cognitive components (Taylor et al., 1990) also all consistently reveal profound impairments in MPTP treated monkeys.

An alternative strategy that avoids the debility and welfare concerns of the bilateral lesions sustained after MPTP administration i.p. has been the development of unilateral lesion models for primates. The advantage of the unilateral lesion, even more so in primates than in rodents, is that the animals are able to feed and groom themselves, and remain healthy throughout the period of experimental evaluation of movement deficits and recovery. Unilateral lesions of the dopamine neurones can be made in primates, as in rats, by intracerebral administration of the conventional toxin, 6-OHDA (Morihisa et al., 1984; Apicella et al., 1986; Annett et al., 1990a). Stereotaxic injection of 6-OHDA unilaterally into the nigrostriatal pathway induces an effective destruction of the dopamine cells in the ipsilateral substantia nigra and degeneration of the dopamine projection to the striatum (Apicella et al., 1990; Annett et al., 1992; Roeling et al., 1995). These lesions produce a well-characterized pattern of impairment involving postural and head bias, turning in response to amphetamine and apomorphine, neglect of contralateral space and side of the body, and impairments in reaching and manipulating food and other objects (Annett et al. 1990b, 1992, 1999; Apicella et al., 1990; Emborg-Knott and Domino, 1998; Henderson et al., 1998), for example in a primate equivalent of the staircase test, first developed for rodents (Marshall and Ridley, 1996; Henderson et al., 1998).

Stereotaxic lesions work well for small primate species, such as the marmoset, but require surgical imaging to achieve accurate placement in larger primates. However, effective unilateral nigrostriatal lesions avoiding the need for stereotaxic surgery can be achieved by recruiting the relative lateralization of the arterial blood supply to the brain. Injection of MPTP into one carotid artery yields profound depletion in dopaminergic neurones in the ipsilateral hemisphere, and unilateral movement disorder on the contralateral side (Bankiewicz et al., 1986; Joyce et al., 1986). These larger monkeys then exhibit a similar profile of movement impairments to that seen in smaller 6-OHDA

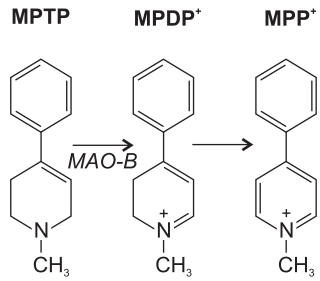

Fig. 9. Chemical structure of the synthetic neurotoxin 1-methyl-4-phenyl-tetrahydro-pyridine (MPTP) and its metabolism, with monoamine oxidase B as substrate, via MPDP+ to the methyl-phenyl-pyridinium ion (MPP+), which is the active toxin. (For further details see Feldman (1997).)

lesioned monkeys, particularly affecting body posture and bias, use of the contralateral limb, and responding to contralateral space. The contralateral deficit involved all the key features of the Parkinsonism including akinesia, rigidity and tremor (Chen et al., 1991), and is responsive to treatment by L-DOPA (Kurlan et al., 1991a; Camarata et al., 1992; Domino and Ni, 1998) or STN stimulation (Benazzouz et al., 1996).

As in rats, analysis of the contralateral neglect exhibited by the hemiparkinsonian monkeys when food is presented in the contralateral field suggested that their deficit was one that involved a failure to initiate contralateral responses ('unilateral hypokinesia') rather than one of sensory detection or attention in contralateral space (Bankiewicz et al., 1991a). Thus, for example, when trained to press levers to achieve food reward, the animals show marked decline in use of the contralateral hand (Brooks et al., 1987; Ellis et al., 1992), and kinematic movement analysis of trained reaching indicates that the monkeys show distinct bradykinesia with slowing of movement and increase in reaction times (Camarata et al., 1992). With the intracarotid injection approach, the deficit is typically stable because of the ability to achieve very extensive ($>95\%$) dopamine lesions unilaterally while the animal remains in good general health (Brooks et al., 1987; Bankiewicz et al., 1991b; Chen et al., 1991; Emborg-Knott and Domino, 1998). Other studies have reported a significant degree of spontaneous recovery (Kurlan et al., 1991b) but are associated with smaller initial deficits and presumably attributable to less complete initial lesions.

MPTP toxicity in rodents. The discovery of MPTP toxicity in man has in parallel resulted in a detailed analysis of the mechanisms of toxicity in vitro and in rodent models (Feldman et al., 1997; Przedborski and Vila, 2001). The MPTP is not by itself an active compound but is converted enzymatically by monoamine oxidase (MAO) in glial cells to the methyl-phenylpyridinium ion, MPP+, which is toxic by disrupting cellular ATP production and increasing superoxide free radical formation (see Fig. 9). MPP+ is then taken up into the neurones via active dopamine and noradrenaline uptake channels, leading to its selective accumulation in catecholamine neurones and correspondingly its selective toxicity for these neuronal systems. The ability to convert MPTP to MPP+ is specific to the B isoform of the MAO. However, there are marked species differences in the relative abundance of these isoforms; whereas MAO-B is abundant in mice and primates (including man), rats and guinea pigs predominantly express the A isoform, rendering these species relatively insensitive to the toxicity of MPTP (Boyce et al., 1984; Chiueh et al., 1984; Heikkila et al., 1989). Nevertheless, if we bypass the limiting MAO-regulated step in metabolism, rats do remain equally susceptible to direct

administration of MPP+ (Heikkila et al., 1985b; Sirinathsinghji et al., 1988). Conversely, selective blockade of the MAO-B inhibits the toxicity of MPTP in mice and primates (Chiueh et al., 1984; Cohen et al., 1984; Hallman et al., 1984; Heikkila et al., 1984), which has provided the basis for the proposition for use of the selective MAO inhibitor, deprenyl, in PD. Although initial studies of deprenyl in patients appeared to slow progression of the disease and prolong lifespan (Birkmayer et al., 1985; Tetrud and Langston, 1989; the Parkinson Study Group, 1992), subsequent studies have suggested that neuroprotective effects are marginal (Kieburtz et al., 1994; Shoulson et al., 1998; the Parkinson Study Group, 1993) and any beneficial effect of deprenyl is most likely attributable to dopaminomimetic rather than neuroprotective actions of the drug (LeWitt, 1993).

Since mice but not rats exhibit clear neurotoxicity in response to MPTP, this species has been very widely used for studies of mechanisms of toxicity, neuroprotection and treatment in vivo (Heikkila et al., 1989). Thus, 5–10 daily injections of MPTP in mice result in a selective and relatively complete loss of dopamine neurones from the substantia nigra, sparing VTA and locus coeruleus, a corresponding loss of dopamine, DOPAC and HVA from the striatum, and expressed behaviorally as a marked hypokinesia (Heikkila et al., 1989). The resulting behavioral syndrome in mice includes akinesia, rigidity, tremor, gait and postural disturbances, and a recent review summarizes more than a hundred studies using a variety of behavioral tests to characterize the different symptoms in considerable detail (Sedelis et al., 2001). Nevertheless, the MPTP syndrome in mice is in practice very similar to that already described for bilateral nigrostriatal lesions in rats (Section 3).

As in monkeys, the MPTP symptoms in mice can be alleviated by L-DOPA (Fredriksson et al., 1990; Rozas et al., 1998) and toxicity can be blocked by deprenyl and other MAO-B inhibitors (Heikkila et al., 1985a; Sundström and Jonsson, 1985). Moreover, deprenyl can rescue dopamine neurones up to 72 h after the MPTP treatment (Tatton, 1993). Further analysis of the neurotoxic process in mice suggest several distinct aspects of MPTP toxicity at the neuronal level, involving first the induction of ATP depletion and oxidative stress leading to a failure in cellular energy production, second the recruitment of molecular pathways, such as those involving Bax and bcl-2, and third an amplification of the neurodegenerative insult involving prostaglandin-mediated inflammation (Przedborski and Vila, 2003). This, then, suggests several different levels at which the cascade of toxicity may be addressed. Thus, the mice treated with nitric oxide synthase (NOS) inhibitors or depleted of neuronal NOS show reduced susceptibility to MPTP (Jenner, 1998). Similarly, since apoptosis appears as a final common pathway in the MPTP model, small antiapoptotic molecules may be neuroprotective in PD (Waldmeier et al., 2001), supported by the ability of CGP3466B to protect mouse nigral neurones from MPP+ toxicity in vitro (Waldmeier et al., 2000) and from MPTP in vivo (Waldmeier et al., 2001). These studies clearly contribute to the current active search for both antioxidative and antiapoptotic strategies for treatment in human PD (Dunnett and Björklund, 1999).

5.2. METHAMPHETAMINE TOXICITY

Although most familiar as indirect agonists, repeated very high doses of amphetamines, can be toxic. The most potent of these is methamphetamine. Thus, four or more repeated

doses of 7.5–10 mg/kg methamphetamine, can induce significant depletions of forebrain dopamine in rats (Wagner et al., 1979; Ryan et al., 1990), mice (Johnson et al., 1992; Kita et al., 1998a) and monkeys (Seiden et al., 1976; Preston et al., 1985). Thus, repeated injections of methamphetamine in mice produce substantial (typically 50–75%) depletions of dopamine in the neostriatum, accompanied by a parallel decline in the metabolites DOPAC and HVA, and reductions in TH activity. The depletions are typically greatest in the neostriatum, whereas levels in other forebrain areas, including ventral striatum, frontal cortex, amygdala and hypothalamus are generally less extensively depleted or remain unaffected. Depending upon the size and distribution of doses, amphetamine-induced depletions in dopamine can be relatively long lasting (Wagner et al., 1980; Preston et al., 1985) or may exhibit substantial recovery (Friedman et al., 1998; Cass and Manning, 1999). The biochemical loss is associated with a loss of dopamine terminals in the striatum and the frontal cortex, and of postsynaptic binding sites (Ricaurte et al., 1982; Ryan et al., 1990). By contrast, reports of methamphetamine-induced loss of TH-immunoreactive dopamine neurones themselves are more variable, with some studies reporting no loss (Seiden and Ricaurte, 1987; Harvey et al., 2000), whereas others report substantial loss of TH-immunoreactive profiles of up to 40% in mice (Hall et al., 1996; Sonsalla et al., 1996) and 78% in rats (Trulson et al., 1985). However, one study of cell counting, based on cresyl violet labeled neurones revealed no loss (Ricaurte et al., 1982), so it needs to be determined whether the loss seen in TH staining is due to an actual loss of nigral dopamine neurones or is dependent upon loss of enzyme activity in otherwise surviving cells. This could be resolved by back-labeling cells with flurogold prior to methamphetamine treatment, but this has not (at least to my knowledge) been reported.

It has been proposed that the dopamine impairment is due to amphetamines inducing toxic radical formation and terminal degeneration in the striatum (Gerlach and Riederer, 1996; Seiden and Sabol, 1996; Huang et al., 1997b), since the biochemical loss is dramatically reduced by a variety of antioxidant treatments (De Vito and Wagner, 1989b; Itzhak and Ali, 1996; Imam et al., 1999), and is exacerbated by treatments that block intrinsic antioxidant processes (Imam et al., 1999). Dopamine neurones exhibit a variety of regulatory mechanisms for neutralizing free radicals (Dunnett and Björklund, 1999), and these may therefore protect against the toxic consequences of their formation by amphetamines. Thus, if the ability of the cells to handle oxidative stress is reduced by depletion of dietary selenium, methamphetamine treatment is now seen to induce loss of TH cells from the substantia nigra, in addition to exacerbating the biochemical depletion (Kim et al., 2000b).

Methamphetamine treatment, as a model of partial depletions has been the subject of a relatively large number of behavioral analyses, in part because of the relevance of this drug to human abuse and addiction. In the acute period, 1–6 days following treatment, activity levels are raised and the animals show an increase in drinking and locomotor activity, in particular during the dark cycle (Kita et al., 1998b). Thereafter, 'neurotoxic' doses of methamphetamine produce relatively few long-lasting motor impairments (Seiden et al., 1993; Gerlach and Riederer, 1996), perhaps because of the relatively incomplete depletions that result. Thus, the animals show no lasting impairments in rotarod performance, T maze alternation, passive avoidance or Morris water maze learning (Walsh and Wagner, 1992; Friedman et al., 1998; Schröder et al., 2003). Nevertheless, detailed analysis does reveal some lasting deficits in more sensitive tests of active avoidance, beam balance (Walsh and Wagner, 1992), reaction time performance (Richards et al., 1993) and novelty

discrimination (Schröder et al., 2003). Although spontaneous locomotor responses are normal, the animals show a blunted response to the stimulant effects of amphetamine or apomorphine and a lower threshold of response to haloperidol (Lucot et al., 1980). Similarly, although the animals were neither aphagic nor adipsic, and baseline consumption of sweetened milk was unaffected, they were more tolerant to the anorectic effects of amphetamine (Bittner et al., 1981; De Vito and Wagner, 1989a).

5.3. OTHER TOXIN MODELS

The discovery of MPTP toxicity heightened support for an environmental hypothesis of the causation of PD, and a wide variety of other potential, natural, industrial and synthetic toxins have been proposed and explored (Tanner, 1989; Lockwood, 2003). Correspondingly, a wide range of compounds related to agrochemical and industrial activities, such as manganese, cobalt, rotenone, paraquat-related compounds, 3-nitrotyrosine and many others have been shown to induce akinetic syndromes in mice (Betarbet et al., 2002). However, although these inform about neurotoxicological issues that may have a bearing on the aetiology of PD and related Parkinsonian syndromes in man, they have not been particularly informative about functional organization of nigrostriatal systems per se, the topic of the present chapter, and are therefore not considered further here.

6. GENETIC MODELS

6.1. SPONTANEOUS MUTATIONS

A number of mutations affecting brain dopamine systems have arisen spontaneously in the rats and mice, have been identified, and have been subsequently maintained as mutant strains.

Weaver (wv^{-}/wv^{-}) mice. The longest known and the most widely studied of mutant strains affecting the dopamine system is the Weaver mutation. First described in 1964 (Lane, 1964), the $wv^{-/-}$ mutation produces a marked ataxia in mice, which in early studies was attributed predominantly to degeneration in the cerebellum (Rezai and Yoon, 1972; Rakic and Sidman, 1973; Sotelo and Changeux, 1974). However these animals also exhibit degenerative changes in the hippocampus (Sekiguchi et al., 1995) and substantia nigra (Roffler-Tarlov and Sidman 1978; Schmidt et al., 1982; Triarhou et al., 1988a).

The dopaminergic degeneration in particular has been proposed as a good model of selective nigrostriatal degeneration (Maharajan et al., 2001) and has been studied in detail. Thus, ventral mesencephalic dopamine neurones are born and develop normally between E11–E15 in the developing Weaver embryo, and the mice are born with a normal complement of dopamine neurons, but then these cells atrophy and die during the weanling period, with a loss of 42% nigral cells by postnatal day 20, and reaching almost 70% by three months of age (Triarhou et al., 1988b). Dopamine cell loss is seen in all areas of the ventral midbrain, but occurs earlier and to a somewhat greater extent from the substantia nigra (A9) than from the VTA and retrorubral areas (A10 and A8, respectively; Triarhou et al., 1988b). Mature animals exhibit a parallel substantial 70% loss of dopamine innervation from the neostriatum, nucleus accumbens and frontal cortex, along with a comparable decline in total TH activity and its metabolites (Schmidt et al., 1982; Reader et al., 1999). Loss of innervation in all terminal areas, not just the striatum, is

accompanied by a compensatory upregulation of dopamine turnover presynaptically (Reader et al., 1999), and a striatal increase in the sensitivity of post-synaptic D2 receptors (Kaseda et al., 1987; Panagopoulos et al., 1993), similar to that seen after nigrostriatal lesions in adult animals (see Section 3.3), and an increase in serotonin concentration and turnover (Triarhou and Ghetti, 1991; Stotz et al., 1993, 1994; Stotz-Potter et al., 1995; Reader et al., 2001), similar to that seen after dopamine depletion from the striatum following neonatal lesions (see Section 3.4).

The behavioral phenotype of the Weaver mutant is characterized by a severe and progressive syndrome that includes motor, spatial and memory deficits (Triarhou, 2002). In particular the movement disorder involves an ataxia including instability of gait and poor limb coordination, which is most probably of cerebellar origin (Lane, 1964; Lalonde, 1987; Triarhou, 2002). In addition, the mice show other deficits more akin to a dopaminergic dysfunction, such as reduced locomotor response, rearing and exploration in the open field, impaired beam balance, hind paw clasping and slower swimming (Lalonde and Botez, 1986; Triarhou, 2002). Moreover, their responses to dopaminergic drugs reflect the profile expected after nigrostriatal lesions, viz. a reduced locomotor response to amphetamine and a heightened response to apomorphine (Schmidt et al., 1982). Deficits in motor coordination when swimming, resting and intention tremors (Lalonde, 1986; Triarhou, 2002) may involve either system.

Further information on the extent to which cerebellar and basal ganglia systems underlie the movement disorder of Weaver mice comes from reparative studies. On the one hand, motor deficits in the Weaver mice can be alleviated with grafts of nigral dopamine cells into the striatum (Triarhou et al., 1986, 1995) but not by cerebellar grafts in the cerebellum (Triarhou et al., 1987; Triarhou 1996), and it has also been possible to rescue the nigra dopamine cells from developmental degeneration in Weaver mice by various neuroprotective agents that are known to protect dopamine systems, such as ganglioside GM1 and the trophic factor GDNF (Schneider et al., 1994; Broome et al., 1999), supporting a dopamine-mediated view of the motor disorder. On the other hand the akinetic phenotype has not been alleviated by L-DOPA (Muroga et al., 1982). Moreover, as Purkinje cells progressively die in the mutant developing cerebellum, remaining Purkinje cells exhibit markedly abnormal firing in conjunction with abnormal head movements (Grusser-Cornehls, 1995), and removing the cerebellum by neonatal lesion can substantially reduce the development of the mice' abnormal gait and impaired balance (Grusser and Grusser-Cornehls, 1998), suggesting that abnormal cerebellar development and gliosis contribute to a 'gain of function' disorder. The Weaver mutation has recently been identified as involving a mis-sense mutation in the G-protein activated inwardly-rectifying K+ channel, Girk2 (Patil et al., 1995), but the precise mechanisms of cell death remain unclear. In particular, the fact that cell death is by a nonapoptotic mechanism in the Weaver substantia nigra (Oo et al., 1996) but does involve apoptosis in the cerebellum (Harrison and Roffler-Tarlov, 1998) suggests that neurodegeneration in the Weaver brain is not likely to be a unitary process.

The AS/AGU rat. The AS/AGU strain is a spontaneous mutation that developed on an Albino Swiss background within the department of Anatomy at Glasgow University. The AS/AGU gene is recessive, and homozygous mice were noted by a progressive locomotor disorder involving a staggering gait, rigidity and tremor apparent from postnatal 10 (Clarke and Payne, 1994; Payne et al., 1998). Pathologically, the brains are grossly normal but the mice, on histochemical analysis are seen to have a marked disruption in the nigrostriatal dopamine system. Specifically, in mature adult animals, approximately 60%

of TH positive cells are lost from the substantia nigra vs. 25% from the VTA (Clarke and Payne, 1994), which is in turn associated with approximately 35% and 15% loss of postmortem levels of dopamine in the dorsal and ventral striatum, respectively (Campbell et al., 1996, 1997). There seems to be, nevertheless, a much greater loss of functional dopamine available at the synapse, up to 90%, as assessed by extracellular dialysis (Campbell et al., 1998). The molecular deficit has recently been characterized as involving a mutation in the gene for protein kinase Cγ (Craig et al., 2001). Since this molecule is believed to be involved in hippocampal plasticity, the AS/AGU mice were tested for hippocampal LTP, but were found to be quite normal on this measure (Shahraki and Stone, 2002). Conversely, there is good evidence that the movement disorder is instead directly attributable to the disruption of striatal dopamine innervation, since the deficits in locomotor activity, the abnormal righting response, and turning on an inclined ramp are all alleviated by treatment of the mutant mice with L-DOPA or with nigral grafts (Payne et al., 1998).

The circling (ci^{-}/ci^{-}) rat. The circling rat is a mutant strain that arose spontaneously in inbred Lewis rats in the University of Hannover, in which the recessive gene, circling (ci), results in vigorous turning in circles at high rate, similar to that seen in activated rats with unilateral nigrostriatal lesions (Loscher et al., 1996). Whether tested in rotometer bowls, in an open field or in the home cage, these affected mice rotate spontaneously at mean rates of 4–5 turns per minute, consistently in one direction, and are enhanced by treatment with amphetamine, although the rate in short bursts could be very much higher (Fedrowitz et al., 2000). Although the circling appears in bursts, it is not associated with epileptiform discharges, behaviorally or physiologically. Rather, the abnormal movements include gait disturbances and abnormal head movements, and the circling is increased by stress and being placed in a novel environment, which has correspondingly been characterized as a 'hyperkinetic disorder with abnormal lateralization' (Lindemann et al., 2001). The affected rats also exhibit deficits in its skilled paw reaching with the paw ipsilateral to the direction of rotation.

The behavioral asymmetry in circling rats is associated with approximately 30% loss of dopamine and 20% loss of HVA and DOPAC activity in the striatum (but not nucleus accumbens or frontal cortex) in the hemisphere contralateral to the direction of circling, whether determined postmortem (Loscher et al., 1996), or by striatal microdialysis in vivo (Fedrowitz et al., 2000). There is a parallel increase in activity of the dopamine transporter and post-synaptic receptor binding (Richter et al., 1999) that may be a compensatory response to the depletion in dopamine release. Structurally, however, there is no detectable loss in nigral dopamine neurones, or any other gross pathological abnormalities (Richter et al., 1999), suggesting that the dopaminergic asymmetry is due to a biochemical dysfunction pre-synaptically, rather than a primary degenerative change. Moreover, the mutant rats are also deaf and exhibit vestibular deficits, with difficulties in many forms of activity involving balance including swimming, suggesting that the circling in these circling (also known as 'ci2') rats may be due, at least, in part to developmental abnormalities in the vestibular system (Kaiser et al., 2001). In this regard, the same group have recently identified another strain (designated 'ci3') with similar phenotype involving behavioral circling and lateralized dopamine dysfunction, but in which the auditory-evoked potentials and the vestibular function were normal (Lessenich et al., 2001); the evidence, therefore suggests that this particular mutation may indeed be attributable to asymmetry in nigrostriatal dopamine circuit. The nature of the mutation has not yet been reported in any of the circling strains.

6.2. TRANSGENICS AND KNOCKOUTS

The last decade has provided new molecular and genetic tools for the analysis of changes in the dopamine turnover, particularly in the generation of strains of knockout mice that are deficient in various aspects of dopamine neurone development, in the cellular machinery for dopamine neurotransmission, or in other genes associated with parkinsonism in man. As several good recent reviews are available (Jankowsky et al., 2002; Eells, 2003), the present account will focus on the behavioral phenotypes associated with genetic manipulation of dopamine systems of the forebrain.

Specification of dopaminergic neurones. Many of the molecular signals that specify differentiation of precursors during embryonic development into dopamine neurones have now been identified, and their location and sequence of expression are being characterized in some detail (Hynes and Rosenthal, 1999a,b; Goridis and Rohrer, 2002; Burbach et al., 2003). The development of dopaminergic neurones of the substantia nigra is impaired after knockout of many of these key signals. For example, sonic hedgehog (Shh) is inductive for specification of the dopamine neurone phenotype in vitro (Hynes et al., 1995) and its absence in knockout mice results in a profound failure of specification of all neuronal types in the ventral neural tube, with the greatest neuronal disorganization in the presumptive midbrain and forebrain by E11.5 (Chiang et al., 1996). These mutant embryos never develop a discernable substantia nigra and they die in utero, presumably not because of this, but because of a general failure of organization of all midline structures, non-neural as well as neural. However in the developing ventral midbrain, Shh and FGF-8 together trigger a cascade of gene expression that eventually results in the development of a dopaminergic phenotype in the neurones of the ventral mesencephalon (Hynes and Rosenthal, 1999b). Knocking out several of these genes, including engrailed, Lmx1b and Nurr1, has also been shown to result in embryonic death of newly differentiated dopamine neurones (Zetterström et al., 1997; Smidt et al., 2000; Simon et al., 2001; Eells, 2003). Whereas most of these mutations are embryonic lethal, the Nurr1 deficient mice do survive beyond birth. Although the brain and other organs exhibit no gross morphological abnormalities, these mice fail to generate midbrain dopamine neurones, and following birth are hypoactive, and show impaired righting reflex and abnormal limb movements. Moreover, they die within 1–2 days, most likely as a result of their failure to suckle (Zetterström et al., 1997; Saucedo-Cardenas et al., 1998; Le et al., 1999b). By contrast, less impairment is found in the Nurr1$^{+/-}$ heterozygote, in which the animals grow to maturity, exhibit no gross behavioral or pathological abnormalities, and a rather selective nigrostriatal degeneration involving a 'slight decrease' in neurones in the substantia nigra and 39% loss of striatal DA (Zetterström et al., 1997; Le et al., 1999b), although other studies have found no significant change in the striatal dopamine levels either (Le et al., 1999a; Bäckman et al., 2003). Although the heterozygotes show no changes in spontaneous activity, they have an enhanced locomotor response to amphetamine (Bäckman et al., 2003) and an enhanced sensitivity to MPTP toxicity (Le et al., 1999a; see Section 5.3) by mechanisms that are not fully clear.

Dopamine synthesis and turnover. Rather than disrupting the specification of presumptive dopamine neurones per se, an alternative strategy is to knockout key components of the cellular synthesis of dopamine production or turnover. As the rate limiting enzyme in dopamine synthesis is TH, TH knockouts offer an obvious strategy. However, deletion of the TH gene effects adrenergic, including sympathetic as well as

dopaminergic systems, peripherally as well as centrally, and is embryonic lethal (Kobayashi et al., 1995; Zhou et al., 1995). However, in a clever strategy to circumvent this limitation of the general depletion model of all catecholamines, Zhou and Palmiter (1995) have combined the TH knockout with a TH transgenic under the control of the dopamine-β-hydroxylase (DBH) the promoter so that the TH is expressed in putative noradrenaline and adrenaline neurones which can then synthesize their respective transmitters normally, with a failure in synthesis only in the presumptive dopaminergic neurones. These '$DA^{-/-}$' knockouts are born at the expected frequency and contain normally appearing nigral neurones in the midbrain but they fail to synthesize dopamine, are profoundly hypoactive and stop feeding within approximately three weeks after birth (Zhou and Palmiter, 1995; Szczypka et al., 1999). This phenotype is lethal within a few weeks after birth but normal feeding and growth can be rescued, and the motor deficits alleviated, by chronic administration of L-DOPA (Zhou and Palmiter, 1995; Szczypka et al., 1999). Although during its period of activity, L-DOPA induced feeding and activity, to normal levels, it restored brain dopamine levels to only approximately 9% of the normal levels, suggesting a supersensitive response at the receptor level, and they showed a hyperactive response to receptor agonist drugs, but dopamine receptor binding and reuptake was not greatly changed from normal levels in either untreated or L-DOPA treated $DA^{-/-}$ mice (Szczypka et al., 1999; Kim et al., 2000a), suggesting a different mechanism of compensation which is yet to be clarified completely.

Other aspects of the dopaminergic phenotype have been disrupted with deletion of other components of the cellular machinery for dopamine storage, release and re-uptake. Since the dopamine transporter (DAT) allows transmitter reuptake from the synapse after release for pre-synaptic reuse, DAT knockout results in chronically heightened levels of extracellular dopamine, as measured by striatal dialysis or voltammetry, and a corresponding downregulation of presynaptic release (Eells, 2003). As a result, the mice are hyperactive throughout life (Giros et al., 1996; Fumagalli et al., 1998; Spielewoy et al., 2000; Zhuang et al., 2001). In particular, the hyperactivity is more apparent in novel test boxes, where they show an abnormal reaction to novelty and a failure to habituate with familiarization, than in the home cage (Spielewoy et al., 2000; Zhuang et al., 2001), and they show a reduced response or an actual inhibition of activity in response to indirect dopamine agonists such as amphetamine and cocaine (Giros et al., 1996; Zhuang et al., 2001), in contrast to the activating effects of these drugs in normal animals (see Section 2.2). Nevertheless, dopamine antagonists do reverse their hyperactivity (Spielewoy et al., 2000; Ralph et al., 2001). This strange pattern of responses has been interpreted as reflecting a change in the balance between post-synaptic receptors and pre-synaptic autoreceptors, similar to that reported in attention deficit hyperactivity disorder (Zhuang et al., 2001; Eells, 2003). Further characterization of the hyperdopaminergic phenotype of $DAT^{-/-}$ mice has indicated prolonged wakefulness and reduced periods of time spent in particular in 'rapid eye movement' sleep (Wisor et al., 2001), impaired exploratory behaviors in a Y maze (Zhuang et al., 2001), impaired social interaction, maternal function and stress responses (Spielewoy et al., 2000), and deficient sensory gating as measured by reduced PPI in the acoustic startle test (Ralph et al., 2001).

In contradistinction to the hyperdopaminemia of $DAT^{-/-}$ mice, knockout of the VMAT2 gene eliminates vesicular uptake of dopamine in the pre-synaptic terminal, reducing dopamine storage and release (Takahashi et al., 1997; Wang et al., 1997; Eells, 2003). Like the $DA^{-/-}$ knockouts, homozygous $VMAT2^{-/-}$ mice die soon after birth, but the heterozygotes live to adulthood. The heterozygotes show normal

spontaneous locomotor activity and passive avoidance (Takahashi et al., 1997) and exhibit an enhanced locomotor response to amphetamine, apomorphine cocaine and ethanol (Takahashi et al., 1997; Wang et al., 1997). The hedonic value of drugs associated with reward is reduced, as indicated by diminished preference for places associated with amphetamine (Takahashi et al., 1997), and a diminished voluntary consumption of ethanol (Hall et al., 2003).

Knockout of different components of the dopamine processing machinery of cells influences the susceptibility of mice to a variety of toxins. Thus, $DAT^{-/-}$ mice show a reduced sensitivity to MPTP (Gainetdinov et al,. 1997) whereas $VMAT^{+/-}$ mice exhibit an enhanced sensitivity to the toxicity of MPTP and amphetamine (Takahashi et al., 1997; Gainetdinov et al., 1998; Fumagalli et al., 1999) but not to L-DOPA (Reveron et al., 2002).

Dopamine receptors. Whereas knockout of essential components of dopamine synthesis storage and release may be expected to disrupt profoundly all dopaminergic systems of the brain, knockout of individual populations of dopamine receptors may be expected to have more selective effects on behavior, because of potential redundancy and differences in regional distribution and pre- vs. postsynaptic localization. Dopamine receptor knockouts have been of particular interest from a psychopharmacological perspective because, although at least five main types of dopamine receptors have been identified molecularly, drugs selective for each subclass have not been available; rather most dopamine receptor ligands fall into one of two classes, 'D1-like' (acting at both D1 and D5 receptors) and 'D2-like' (acting at D2, D3 and D4 receptors; Feldman et al., 1997). Psychopharmacological studies in mice deficient for individual receptors, therefore, allow the possibility of identifying by exclusion the classes of behavior affected by dopamine transmission that are mediated by each (Sibley, 1999). A comprehensive review of selective dopamine receptor knockout mice from such a perspective has recently been published (Waddington et al., 2001; and see Chapter 3).

The selective dopamine receptor knockout strains show different profiles of effects on spontaneous activities and motor behaviors. Thus, whereas in initial studies, D1 knockouts showed no gross neurological deficits, they did require a supplemented and hydrated diet to thrive. Moreover, although they show normal motor coordination and locomotor activity levels, they exhibit reduced rearing and exploration (Drago et al., 1994). Another strain on a different background has been seen to show a modest increase in activity but a reduction in grooming (Xu et al., 1994). Analysing the phenotype by a more complex 'ethogram' notation, Waddington concludes that D1 knockouts do not show overall increases or decreases in activity per se, but a complex shift between different elements of behavior in the animals' natural repertoire (Clifford et al., 1998). Knockout of the D5 receptor, in the same D1-like class, again produces no gross neurological abnormalities. Although an early report suggested that D5 knockout mice exhibit an increase in spontaneous locomotor activity, and improved performance in motor coordination and balance on a rotarod (Sibley, 1999), more detailed characterization suggested that they are relatively normal on a wide range of spontaneous behaviors including locomotor activity, rotarod, acoustic startle, PPI, elevated plus maze, exploration, water maze swimming and fear conditioning (Holmes et al., 2001). However further physiological analysis has suggested that they do show a primary systemic impairment in sympathetic tone leading to hypertension (Hollon et al., 2002).

Mice with deletion of the D2 receptors have typically exhibited more marked behavioral debility, including reduced locomotion and rearing, catalepsy akin to the

bradykinesia of PD (Baik et al., 1995; Clifford et al., 2000). The mice also exhibit impairment in opiate mediated reward (Maldonado et al., 1997). However, another line of D2 knockout mice showed no similar impairments (Kelly et al., 1998), and cross breeding the two lines on common backgrounds suggested that the profound bradykinetic deficit of the earlier strain may have been dependent upon an interaction with the 129 background rather than to the lack of D2 receptors per se. The D3 mice show an opposite impairment, exhibiting increased locomotor and rearing activity (Accili et al., 1996; Xu et al., 1997), which may be at least in part attributable to a reduced level of anxiety, as assessed in an elevated plus maze, in the mice (Steiner et al., 1997). Finally the D4, like the D2, knockouts show reduced activity and rearing in open field environments (Rubinstein et al., 1997), reduced exploration (Dulawa et al., 1999), and higher levels of fear or anxiety to novelty (Falzone et al., 2002). Thus, rather than distinct patterns of different dopamine receptor depletions affecting discrete aspects of the behavioral lesion and antagonist syndromes, we see instead rather diffuse effects in each knockout line enhancing or reducing animals' general levels of activities and responses to novelty and reward, suggesting involvement of the different classes of receptors in the complementary regulation of motor responsiveness to significant stimuli in the environment. The detailed effects of selective agonist and antagonist drugs on the different transgenic and knockout strains is beyond the scope of the present review, but more detailed synopses can be found in Sibley (1999) and Waddington (2001).

α-*Synuclein*. The final class of transgenic lines relevant to the present review is animals carrying mutations that have been identified to cause PD in rare familial pedigrees with the disease. The first gene found associated with a familial PD was the gene for α-synuclein, in which two missense mutations, (A30P and A53T), have been genetically linked to rare familial forms of the disease in (Polymeropoulos et al., 1997; Kruger et al., 1998). The α-synuclein protein was first identified in amyloid plaques and has been found to constitute an essential component in Lewy bodies (Spillantini et al., 1997). Knockout mice from which the α-synuclein gene is deleted develop normally, show normal nigrostriatal development and normal levels of spontaneous activity, but they exhibit reduced dopamine turnover in the host striatum and a reduced locomotor response to amphetamine (Abeliovich et al., 2000). Conversely overexpression of the wild type human α-synuclein gene in transgenic mice results in a progressive accumulation of α-synuclein and ubiquitin immunoreactive aggregates in neurones in the neocortex, hippocampus and substantia nigra, associated with a loss of dopamine terminals in the striatum and locomotor impairments (Masliah et al., 2000). Expression of either of the PD mutation as opposed to the human wild type of α-synuclein results in a rather similar pattern of pathology involving cellular aggregations of protein but with a variable degree of fibrillary structure as seen in the human Lewy body (Kahle et al., 2000; Masliah et al., 2000; Van der Putten et al., 2000; Giasson et al., 2002; Neumann et al., 2003) and widespread gliosis (Giasson et al., 2002; Lee et al., 2002; Gomez-Isla et al., 2003). Dopaminergic neurones of the substantia nigra are typically not lost (Van der Putten et al., 2000; Gomez-Isla et al., 2003), although some studies report loss of dopamine terminals in the striatum (Masliah et al., 2000). Several of these mutant lines have exhibited impairments in locomotor activity (Giasson et al., 2002; Lee et al., 2002), which at least in the most marked cases appears to be due to widespread cellular dysfunction and collateral degeneration in motor neurones (Van der Putten et al., 2000; Giasson et al., 2002) rather than being selective for the nigrostriatal system. Moreover, in line with cortical and hippocampal pathology, α-synuclein transgenics have been reported as exhibiting impairments in conditioning at

the electrophysiological level (Steidl et al., 2003) although escape learning in the Morris water maze is unaffected (Masliah et al., 2001). Since the A53T mutation in humans represents the normal isoform in mouse, and most synucelinopathies in mouse and man involve the normal or mutant form of the gene, the role of α-synuclein in neurodegeneration in general and dopaminergic degeneration in particular most likely relates to the role of this protein in the brain's handling of oxidative damage and impairments in protein catabolism (Dev et al., 2003), and is therefore less informative for our present purpose exploring the specific functions of dopamine systems per se.

Behavioral effects of transgenic mice that overexpress other *parkin* genes associated with familial PD are yet to be reported.

7. SUMMARY AND CONCLUSIONS

Unilateral and bilateral depletions of the forebrain dopamine systems produce distinctive patterns of motor impairments in a wide variety of animal models. Irrespective of the individual model, we see considerable overlap in the resulting functional syndrome, reflecting the fact that the behavioral impairments are defined by the lost functions associated with the dopamine neuronal substrate rather than distinctive effects of the individual treatments. These have allowed the evolution of a well-defined range of behavioral tests to characterize different aspects of the functional deficit including:

- locomotor activity and the levels of global activation or arousal;
- motivation and regulation of consummatory responses to meet physiological needs such as nutrient intake and fluid balance;
- Motor coordination and balance, under involuntary motor control;
- selection, initiation speed and accuracy in the execution of skilled movements under voluntary control.

From these various analyses, it is clear that dopamine regulation of the striatum does not simply control detailed movement, but is involved in the selection and initiation of appropriate goal directed actions (Dunnett and Robbins, 1992; Robbins and Everitt, 1992), as influenced by motor learning (i.e. the acquisition of skills and habits; Mishkin et al., 1984; Jog et al., 1999), in the context of motivational information related to needs and rewards (Suri and Schultz, 1999). Theoretical formulations of this process have moved away from the neuropsychological theory, although still conceptually useful, to mathematical and neural network modeling (Houk et al., 1995; Servan-Schreiber et al., 1998), which is beyond the scope of the present review.

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CHAPTER VI

Dopamine, motivation and reward

GAETANO DI CHIARA

ABSTRACT

Experimental manipulation of dopamine (DA) transmission by lesions and drugs indicates a role in the acquisition and expression of behavior motivated by conventional and drug reinforcers. This role is related to modulation of ionotropic transmission, mainly in the ventral striatum (n. accumbens/olfactory tubercle), in components of the so-called extended amygdala (central amygdala and bed nucleus of stria terminalis) and in the medial prefrontal cortex. DA release in the n. accumbens elicits an appetitive state (euphoria), facilitates memory consolidation in Pavlovian learning, promotes approach behavior towards novel cues and contexts and mediates the energizing action of conditioned stimuli on instrumental behavior. These functions can be related to differential stimulation of DA transmission by conditioned and unconditioned motivational stimuli in different DA terminal areas. In the n. accumbens shell, DA responsiveness follows Pavlovian rules, being related to appetitive valence, novelty and unpredictability of the stimulus. In contrast, n. accumbens core and medial prefrontal cortex DA responds to both conditioned and unconditioned stimuli in relation to their generic motivational relevance. These properties conform to a role of DA in incentive arousal. This term describes the state induced by DA released in response to motivational stimuli and its widespread influence on behavior: euphoria and elevated mood, facilitation of the acquisition of incentive properties by otherwise neutral stimuli and contexts through Pavlovian associations (incentive learning), facilitation of instrumental and approach behavior by conditioned incentives. Addictive drugs share the ability to stimulate DA transmission in the ventral striatum and in particular in the accumbens shell. Various hypotheses attribute to this property a role in the mechanism of drug addiction. According to the incentive-learning hypothesis, drug addiction is the result of the excessive incentive properties acquired by drug-conditioned stimuli following maladaptive stimulation of DA transmission in the accumbens shell.

KEY WORDS: Dopamine; reward; motivation, incentive; accumbens; learning; food; addiction; sensitization.

1. INTRODUCTION

Early studies on the behavioral effects of systemic DA receptor blockers (neuroleptics) showed that these drugs disrupt instrumental responding for a variety of rewards both

conventional (food, water, sex) and unconventional (drugs, intracranial self-stimulation) (Dews and Morse, 1961). These effects were initially interpreted to be due to an impairment of motor performance (Rolls et al., 1974; Fibiger et al., 1975), a suggestion consistent with the then dominant view about brain DA as being specifically involved in extrapyramidal motor functions related to the basal ganglia. However, a purely motor view would not account for the antipsychotic effects of neuroleptics. In the meantime, a detailed mapping of the projections of DA neurons showed that DA was not confined to striatal territories receiving motor input but extended to areas receiving 'limbic' input, traditionally related to motivation (Ungerstedt, 1971; Lindvall and Bijorklund, 1974) and even to prefrontal cortical areas involved in cognitive and executive functions (Thierry et al., 1973; Berger et al., 1974; Lindvall et al., 1974). This anatomical knowledge was instrumental in the interpretation of mapping studies of the brain sites from which ICSS could be evoked or disrupted. Thus, the discovery that the brain 'pleasure centers' (Olds and Milner, 1954) were strikingly correspondent to the terminal areas of the mesolimbic DA system (Wise, 1978) set the stage for the hypothesis of a role of DA in behavior independent from motor function.

2. GENERAL OUTLINE

The role of DA in motivation is a topic of an immense literature. This makes a review on it necessarily focusing on specific issues, essentially those of interest to the author (and hopefully to the reader).

In reviewing the role of DA in motivation, this chapter will separately consider and compare behavior motivated by conventional (mostly food) and drug reward. The reason for this is that, in addition to obvious similarities, conventional and drug rewards along with the behavior motivated by them show differences that require a distinct interpretation and might be critical for the behavioral disturbances that characterize drug addiction.

This review can be divided into four main sections. The first section provides the anatomical background and defines the basic behavioral terminology. The second section reviews studies on the effect of experimental manipulation of DA transmission on conventional and drug reward. The third section reviews the changes in DA transmission in specific brain areas and in the activity of DA neurons in response to conventional as compared to drug rewards, and to stimuli conditioned by them as well as during behavior reinforced by these stimuli. The fourth section provides an interpretative framework of the studies analytically reviewed in the previous sections.

3. ANATOMICAL BACKGROUND

The terminal DA areas more directly involved in motivation belong to the striatal and the archistriatal areas (central nucleus of the amygdala and bed nucleus of the stria terminalis) that constitute Heimer's 'extended amygdala' (Alheid and Heimer, 1988; Heimer et al., 1991).

On the basis of connectional, histochemical and comparative anatomical grounds, the striatal complex has been usefully distinguished into three sectors, a medio-ventral, limbic sector, including the NAc shell and core and the olfactory tubercle and corresponding to the ventral striatum according to Heimer and Wilson (1975); an intermediate, associative

sector and a dorso-lateral, sensory-motor sector (Joel and Weiner, 2000; Riedel et al., 2002).

Experimental as well as correlative studies point to the nucleus accumbens (NAc) as the critical site of origin of the role of DA in motivation. This area has been subdivided into three main subterritories, the shell, the core and the rostral pole (Zahm and Brog, 1992; Zahm and Heimer, 1993). However a shell and a core component can be distinguished also in the rostral pole on the basis of combined calbindin (a core marker) and calretinin (a shell marker) immunoreactivity (Riedel et al., 2002). Although calcium binding proteins provide the best means for shell/core distinction, from a practical point of view, it should be pointed out that while the NAc shell can be distinguished from the adjacent core also on unstained sections, being separated from it by a wall-like formation – the NAc core merges continously into the dorsal striatum.

Kelley has recently summarized the connections of the NAc in relation to feeding behavior (Kelley, 2004) (Fig. 1).

The NAc receives brainstem information related to taste and visceral functions through direct input from the nucleus of the solitary tract to the medial shell as well as indirect input from the gustatory cortex to the lateral shell and core via parabrachial projections to the gustatory thalamus (Ricardo and Koh, 1978; Saper, 1982). Additonal taste information is relayed to the NAc from the basolateral amygdala, that integrates taste

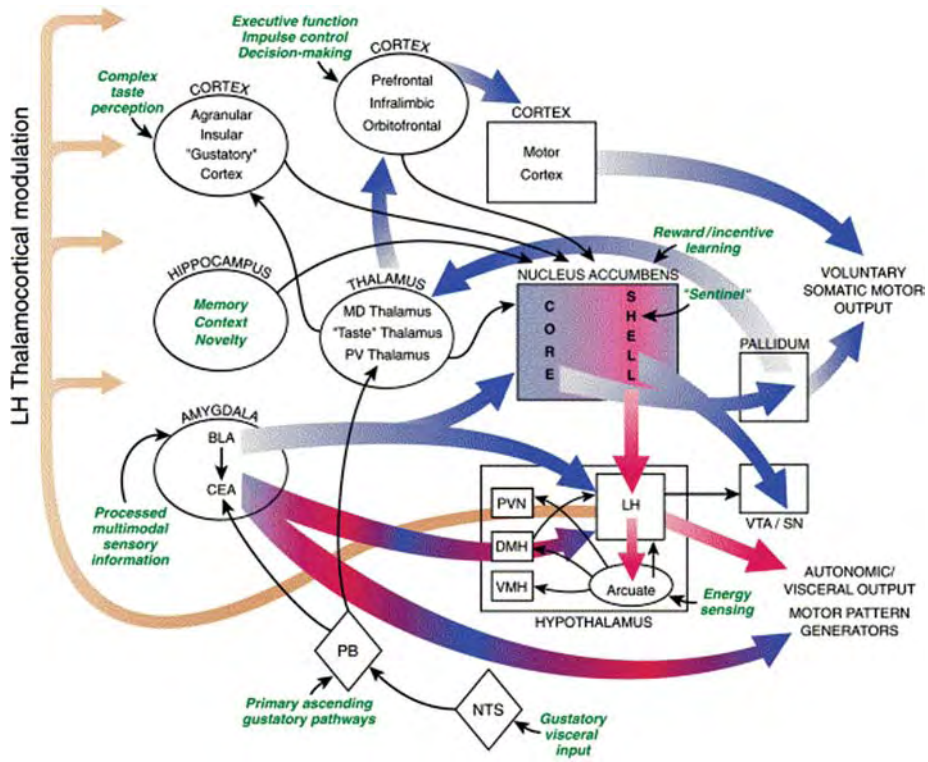

Fig. 1. Diagram of the connections of the ventral striatum involved in food-motivated behavior (from Kelley (2004), with permission).

information from the gustatory cortex with multimodal sensory input (McDonald and Jackson, 1987). The NAc shell receives excitatory input from the ventral subiculum relaying spatial information important for the search and approach of food. DA input from the VTA is under control the central nucleus of the amygdal which receives visceral autonomic input arising from the solitary tract nucleus, via the parabrachial nucleus (McDonald and Jackson, 1987) and cortical input from gustatory cortex (McDonald et al., 1999). The central amygdala in turn widely affects the CNS function by its projections to the nuclei of origin of forebrain nonspecific-projection systems, namely the noradrenergic locus coeruleus, the serotonergic raphè nuclei and the cholinergic nuclei of the basal forebrain (Price, 2003; Fudge and Haber, 2000). These systems are traditionally regarded to make up, together with mesolimbic and mesocortical DA neurons, the forebrain arousal system (Robbins and Everitt, 1987).

Efferent projections from the NAc core and shell topographically terminate in the lateral and medial ventral pallidus respectively, but in addition the NAc shell targets the lateral hypothalamus, central grey and nucleus of the solitary tract (Heimer et al., 1991).

The medial accumbens shell, via its reciprocal connections with the lateral hypothalamus (Heimer et al., 1991; Brog et al., 1993), receives information on the internal state from the arcuate nucleus of the hypothalamus and in turn modulates the release of fixed motor patterns of feeding after local infusion of GLU-antagonists and GABA agonists (Maldonado-Irizarry et al., 1995).

These connections show that the NAc shell integrates sensory, spatial, visceral and memory information related to food reward with aminergic modulatory input, thus driving the hypothalamic and brainstem motor centers independently from a motor cortical relay. In contrast, the NAc core integrates basolateral amygdala input related to the affective value of discrete sensory information with medial prefrontal and anterior cingulate cortex input, thus tracking instrumental contingency and discriminative sensory properties of CS and relaying them into a classical striatocortical loop (Alexander et al., 1990). These anatomical differences are consistent with the involvement of the NAc shell in activational and incentive aspects and of the NAc core in directional and instrumental aspects of motivation (Corbit et al., 2001).

The NAc shell is heavily interconnected with the so-called medial extended amygdala (Alheid and Heimer, 1988; Heimer et al., 1991). This complex is made up of a number of areas such as the bed nucleus of stria terminalis and the central amygdala, heavily innervated by DA projections and organized in a striato/pallidal-like fashion (Cassel et al., 1999). These areas show some similarities with the NAc shell that include an extensive interconnection, sharing of projections to the lateral hypothalamus and visceral brainstem centers, and a convergence of the DA and the NA projections (Zahm et al., 1999). Although some authors refer to the NAc shell as part of the extended amygdala, the NAc shell was originally kept distinct and eventually viewed as a transition area between the extended amygdala and the ventral striatum (Zahm, 1998). More recently, on the basis of the comparative distribution of calcium binding proteins and peptides, the terminal DA areas of the extended amygdala have been further separated from the striatum and specifically from the NAc shell and grouped with the lateral septum into a parastriatal complex (Riedel et al., 2002).

A detailed account of the areas involved in motivation is beyond the scope of this chapter and has been provided by Cardinal et al. (2002).

4. TERMINOLOGY

In reviewing the role of DA in motivation we, like other authors, have felt the need to define the main constructs utilized to describe behavior. The reason for this is that the meaning attributed to these constructs by authors throughout the literature has not been fully consistent.

4.1. MOTIVATION, REWARDS, INCENTIVES AND REINFORCERS

The concept of motivation is closely linked to the principle that life has the goal of self-perpetuating and that organisms reproduce for the survival of their own species. The substrate of motivation is hard-wired by evolution in the brain of organisms, including man. Organisms are provided with the innate ability to code the intrinsic biological value of stimuli and to respond in a manner consistent with that code (Glickmann and Shiff, 1967). Thus, certain stimuli, such as the taste of a sweet, the smell of a female, the cry of a predator, evoke behaviors that, depending on the motivational valence of the stimulus, result in approach to or avoidance of the stimulus. These responses are not the result of learning by experience of the consequences of the stimuli or of sheer imitation of the behavior of conspecifics. They are in fact unconditioned primary responses.

Motivation broadly refers to those behavioral processes by which organisms emit responses to stimuli (motivational stimuli) in relation to their consequences in terms of survival of the self and species. This is accomplished by learning of predictive relationships (contingencies) between salient stimuli and biologically meaningful ones, and also between responses and their consequences (outcomes) (Mackintosh, 1974). The learning of these contingencies enables the subject to actively promote by its actions the occurrence of biologically valuable events (instrumental action).

A fundamental property of motivational stimuli is their 'motivational valence'. This property determines the direction of the response in relation to the stimulus. Stimuli with a positive motivational valence elicit approach; stimuli with a negative motivational valence elicit aversion. Motivational valence can be either unconditioned or conditioned, as a result of learning of its association with a primary motivational stimulus or with the outcome of a motivated response.

Behavior motivated by natural stimuli can be distinguished into 'appetitive' (or preparatory) and 'consummatory' phases; these phases can be regarded as patterns of responses to specific classes of motivational stimuli (Woodworth, 1918; Konorski, 1967). On this basis, two main classes of positive motivational stimuli can be distinguished: 'incentives' and 'rewards'. Incentive stimuli (Bolles, 1975; Bindra, 1976) are operative in the appetitive phase, while the rewarding stimuli act in the consummatory phase of motivated behavior. Incentive stimuli are experienced through sensory modalities (olfactory, auditory, visual) that do not require contact with the source of the stimulus and allow its detection from a distance. Accordingly, incentives are instrumental for reaching the goal but are themselves not the goal of motivated behavior. Rewarding stimuli are experienced through sensory modalities involving contact (taste, tactile, proprioceptive, visceral) and are unconditionally predictive of biological outcomes that provide the final goal of motivated behavior. Because of their integrated functions, rewards and incentives often coexist, being embedded into stimulus complexes (objects, e.g. food, water, drug or organism, e.g. a sexual partner). For example, the complex stimulus of food has conditioned incentive properties (smell) as well as unconditioned

rewarding properties (taste). Although some authors distinguish between the primary (unconditioned) and the secondary (conditioned) rewards, we prefer to reserve the term 'reward' for consummatory unconditioned stimuli.

Incentives are commonly attributed to at least two properties: (1) a directional property that promotes responses directed towards the incentive itself and, through it, towards the reward to which the incentive is related, either conditionally or unconditionally; (2) an activational property consisting of a state of activational arousal (incentive arousal) that increases in a nonspecific manner the incentive properties of other stimuli present in the environment but not necessarily related to the reward to which the triggering incentive has been conditioned. This arousing property of incentives can explain their ability to trigger, under appropriate conditions, the repetitive and excessive emission of behaviors that are part of the species repertoire (adjunctive or displacement behavior) (Falk, 1977; Killeen et al., 1978). Incentive stimuli can be either unconditioned or conditioned as the result of a process of incentive learning.

According to Skinner, a reinforcer is a stimulus capable of strenghtening responses upon which it is contingent (i.e. to which it follows). In this way, reinforcing stimuli are defined post hoc, i.e. on the basis of their ability to increase response emission, without making any assumption on the properties that make them reinforcing. According to the Skinnerian definition, any stimulus can become reinforcing, provided it is made repeteatedly contingent upon a given response. This prediction, however, turns out to be fallacious, as stimuli are differentially capable of acting as reinforcers in a species-specific manner as dictated by their motivational relevance. This observation provides a link between reinforcers and motivation and leads to view reinforcement as a particular case of motivation. Thus, depending on their motivational valence, reinforcers can promote responses that favor (positive reinforcers) or reduce (negative reinforcer) the probability of their presentation. Rewards are able to increase the strength of behavioral responses upon which they are contingent. Therefore, rewards are reinforcers. However, reinforcers are not necessarily rewards. Moreover, reinforcers can be primary, unconditioned or secondary, conditioned stimuli.

4.2. PAVLOVIAN INCENTIVE LEARNING AND RESPONDING

Pavlovian learning provides the opportunity for extending the biological response-code of a primary stimulus (reward or punisher) to other stimuli by the learning of stimulus-reward contingencies (Pavlovian learning) (Mackintosh, 1983). By this mechanism, novel salient stimuli that reliably predict the occurrence of an unconditioned stimulus (US) acquire conditioned response-eliciting properties (CR) consistent with the valence of the US, thus becoming conditioned stimuli (CS). According to Konorski (1967), CS, depending on their nature, can elicit conditioned consummatory or preparatory responses. Conditioned consummatory responses are phenomenologically similar to the correspondent unconditioned response (UR) and can be understood as the result of the excitation by the CS of a representation of the US. Conditioned preparatory responses, instead, are not specific to a given US since, irrespective of it, they consist of flexible patterns of orienting, approaching and exploring the CS. These typically incentive responses, in contrast to consummatory CR, are quite different from the response to the US (UR). For this reason, it is difficult to explain their emission as the result of the direct excitation by the CS of the representation of the US. According to Konorski (1967), these preparatory (incentive) responses to the CS can be explained to be due to the excitation of a motivational system

common to different US. Thus, as a result of the association with the US, the representation of the CS establishes a connection with this motivational system (Pavlovian incentive learning) (Dickinson and Balleine, 1994) thus acquiring the ability of inducing preparatory-incentive responses (Konorsy, 1967). An important property of incentives is that of increasing the emission of responses instrumental to the presentation of the reward to which they have been conditioned. Thus, incentives acquired through Pavlovian (stimulus–reward) associations are capable of energizing primary reinforcement (Estes, 1943; Bower and Grusec, 1964; Trapold et al., 1968; Mellgren and Ost, 1969; Lovibond, 1983). This property has been termed 'transfer from Pavlovian to instrumental responding' (PIT) (Dickinson and Balleine, 1994). Two forms of PIT can be distinguished depending on the fact that the Pavlovian stimulus increases the response for the same outcome to which it has been conditioned (specific PIT) or for a different one (nonspecific PIT) (Rescorla and Solomon, 1967; Colwill and Rescorla, 1988; Balleine, 1994). We regard the nonspecific form of PIT as an expression of incentive arousal. Incentives acquired through Pavlovian contingency learning are also capable of acting as secondary reinforcers, promoting responding instrumental to their own presentation in the absence (extinction) of response reinforcement by the reward.

4.3. INSTRUMENTAL LEARNING AND RESPONDING

Pavlovian incentive learning, apart from increasing, through its activational effects, the probability of encountering a reward present in the environment, does not provide per se organisms with the ability of controlling by their actions the occurrence of biologically significant events. Instead that is what instrumental learning does and what instrumental responding is all about.

Instrumental learning is formally defined as the learning of a stimulus–response contingency. As a result of this learning, the organism emits responses contingent upon the stimulus.

In the past, instrumental responding was explained in a rather mechanistic way as strengthening of the tendency to emit a response to a situational stimulus by its satisfying consequences (Thorndike, 1988) or as simple strengthening of the association between an arbitrary stimulus (S) and a response (R) by its consequences (e.g. feeding) (Watson, 1913; Moss and Thorndike, 1934; Hull, 1943). This modality of instrumental responding might be approximated by the current notion of 'habit responding'.

In the habit modality, response is mainly controlled by stimuli that precede rather than follow it (outcomes) (Dickinson, 1994). As a result of this, devaluation of response outcome fails to impair habit responding. Habit responding takes place as a result of exhaustive training on high ratio schedules or under variable interval schedules where reinforcement is loosely related to response (Dickinson, 1994).

However, under short trials of continuous reinforcement schedules, where every response is reinforced, the responding is tightly controlled by an act–outcome contingency and by a representation of the value of the outcome. The representation of the value of the outcome that motivates instrumental responding is called instrumental incentive value. This value may not coincide with the current hedonic value of the outcome. Updating of instrumental incentive value to the current hedonic value of the outcome requires the reexposure to and the experience of the outcome, a process termed instrumental incentive learning (Dickinson and Balleine, 1994, 1995). The dependence of responding from a tight response-reward contingency would indicate that this form of instrumental responding

(incentive instrumental responding) is controlled by the establishment of a declarative (conscious) representation of the cause–effect relationship between each act and its outcome (Dickinson and Balleine, 1994, 1995); on the other hand, the circumstance that under extinction conditions responding is controlled by the representation of the reward (outcome) rather than by its current hedonic value would indicate that incentive instrumental responding is truly directed toward an abstract representation of the goal (Dickinson and Balleine, 1994, 1995).

Dickinson and associates (Dickinson and Balleine, 1994, 1995) refer to learning of the current value of the outcome as instrumental 'incentive' learning and to the value of the outcome as 'incentive' value.

Thus it happens that the term 'incentive', that we have already seen as being utilized by incentive-motivational theorists (Bindra, 1976) in the context of a procedural, implicit and therefore unconscious form of respose, such as Pavlovian responding, is now utilized in the context of a declarative, explicit and therefore conscious form of response such as act–outcome instrumental responding. This use of terminology is redundant and forces the adding of attributes to the 'incentive' term in order to avoid confusion. Therefore Dickinson and associates distinguish 'Pavlovian' incentive learning from 'instrumental' incentive learning (Dickinson and Balleine, 1994, 1995).

With practice, incentive (act–outcome) instrumental responding is transformed into habit responding based on S-R associations (Dickinson, 1994); this modality ensures responding to stimuli at a speed that would be unattainable by incentive instrumental responding, due to its dependence on outcome. Responding, although impervious to adaptive control by its outcome under the habit modality, can be switched back to the act–outcome modality after repeated failure to meet the requirements of a situational change under the habit modality. This results, after stabilization and practice, in the acquisition of a new habit. In this manner, intentional act–outcome modalities alternate with automatic habit modalities of responding in relation to the changing needs of the external world (Toates, 1998). Such interplay among the different modalities of instrumental responding also applies to addictive behavior (Tiffany, 1990).

5. EXPERIMENTAL STUDIES ON THE ROLE OF DA IN MOTIVATION: METHODOLOGICAL CONSIDERATIONS

As in previous reviews, we have separately considered experimental studies, involving direct manipulation of DA transmission, from correlative studies, involving monitoring of the DA function by electrophysiological, neurochemical and electrochemical means. The evidence provided by these two approaches has been quite different, the first being intended to establish causal relationships, the second to establish temporal realtionships. However, post hoc ergo propter hoc (after it than because of it); therefore, evidence of contingency will strengthen causal relationships drawn on the basis of experimental evidence. In addition, correlative studies provide a mechanistic explanation of the relationships drawn from experimental studies.

Although complementary in principle, experimental and correlative approaches have often provided apparently contrasting results. In the specific case of the role of DA in motivation this might be due to the kind of experimental approach utilized as a basis for comparing experimental and correlative evidence.

Experimental manipulations can result in irreversible inactivation (by surgical ablation, stereotaxically guided lesion, covalent chemical inactivation, genetic deletion, etc) or reversible change (by systemic or local intracerebral drug administration) of a given substrate. The loss of function resulting from irreversible inactivation of a neural substrate, while regarded as the gold standard of evidence for an 'essential' role of that substrate in a given function, has also been the origin of the hottest debates, its main pitfall being the relative nonspecificity, related to lesion of fibers of passage. Introduction of excitotoxins has limited although not completely eliminated this flaw to add a new, more insidious source of nonspecificity, that of transsynaptic and distant degeneration, an aspect poorly considered in studies utilizing excitotoxins as a lesioning tool (Krammer, 1980; Stefanis and Burke, 1996). Another problem with the use of excitotoxins derives from the evaluation of the extent of the lesion (Jongen-Relo and Feldon, 2002).

Studies on the role of DA have taken advantage of the availability of 6-OHDA as a lesioning tool of DA neurons (Ungerstedt, 1968).

Major discrepancies have been found between the results obtained by nonspecific versus DA-specific manipulation of a given area. There are numerous examples of this and only some will be quoted here. One such example involves the effects of reversible manipulation of DA transmission vs. excitotoxic lesion of the NAc and amygdala on Pavlovian versus instrumental learning. While manipulation of DA transmission by infusion of DA agonists indicates the NAc shell rather than the NAc core as a site critical for discriminated Pavlovian learning (Phillips et al., 2003), the opposite has been reported after excitotoxic lesions (Parkinson et al., 1999). A further example along this line is provided by the observation that while nonspecific inactivation of the NAc by either reversible (local lidocaine) or irreversible (excitotoxic lesions) does not affect reward-related shortening in reaction time of instrumental responding, consistent impairment is induced by reversible manipulations of DA or glutamate transmission (Giertler et al., 2004). Therefore, caution should be exercised in making inferences over the role of DA in a given area on the basis of evidence provided by nonspecific lesions or inactivations of that area.

5.1. DOPAMINE, REWARD AND HEDONIA

The first indication that there was more than an impairment of motor function in the disruption of instrumental responding by neuroleptics came from the observation that these drugs typically induce a delayed, within-session decrement of the rate of lever pressing in continous reinforcement schedules (Wise, 1982). This peculiarity appeared to be a general one, as it applied to responding for intracranial self-stimulation, as well as for conventional (water, food) and psychostimulant reward (see Wise et al., 1978; Wise, 1982; Salamone, 1987, for an account and discussion of these studies). The delayed character of the action of neuroleptics on responding, while ruling out a performance effect, also made it similar to the effect of nonreinforcement, i.e. of extinction, thus providing the basis for the hypothesis that neuroleptics impair responding by blunting the hedonic impact of rewards (original anhedonia hypothesis) (Wise et al., 1978; Wise, 1982).

Soon after the formalization of the original anhedonia hypothesis, however, Phillips and Fibiger (1979) reported that neuroleptics reduce responding also when given under extinction; moreover, Gray and Wise (1980) observed that DA receptor blockers impaired

responding on variable interval schedules even before the first reward had been earned, i.e. before reinforcement had taken place. Superimposed on this effect was a progressive reduction of responding. On this basis Gray and Wise (1980) hypothesized that DA mediates the incentive–motivational properties of both primary, unconditioned reinforcers (rewards) and secondary (conditioned) ones and accordingly revised the original anhedonia hypothesis to an incentive–motivational one. As stated by Wise (1982) '*dopaminergic impairment disrupts first and most strongly the motivational arousal function of external rather than internal stimuli*' and, even more explicitly, '*I am suggesting that reinforcers and their associated environmental cues lose their sensory impact in terms of arousal function but not in terms of cue function*'. The idea that DA mediates the activational rather than the directional aspects of response to reinforcers has been later included in many accounts of DA function (Salamone, 1987; Robbins et al., 1989). This revised anhedonia hypothesis took from the incentive-motivational theories of Bindra (1974, 1978) the notion that incentives acquire not only the response-eliciting but also the hedonic properties of the reward to which they have been conditioned.

However, even an incentive–motivational version of the anhedonia hypothesis could not account for the observation of Phillips and Fibiger (1979), replicated by various studies (Gray and Wise, 1980; Mason et al., 1980; Tombaugh et al., 1980; Feldon et al., 1988), that neuroleptics impair nonreinforced responding to a larger extent than reinforced responding. In fact, as argued by Phillips and Fibiger (1979), if indeed neuroleptics impair both the impact of reward (abolished under extinction) and that of conditioned incentives (preserved under extinction), they should similarly impair reinforced and nonreinforced responding to a similar extent.

Among the early studies on the mechanism of neuroleptic-induced impairment of instrumental responding. Those by Beninger and Phillips (1980) deserve an important place, where they first suggested that neuroleptics impair Pavlovian incentive learning. Thus, it was argued that a progressive loss of incentive properties of Pavlovian stimuli on instrumental responding as a result of impairment of Pavlovian incentive learning could explain the within-session impairment of responding induced by neuroleptics.

As a result of these early studies, therefore, four main hypotheses were considered to explain the effect of DA-receptor blockers on instrumental behavior: (1) Blunting of the rewarding properties of primary reinforcers (original anhedonia hypothesis); (2) Loss of the incentive-motivational and arousal properties of primary reinforcers (rewards) and of stimuli conditioned to them (incentives) (revised anhedonia hypothesis); (3) Impairment of performance and sensory-motor functions; (4) Impairment of Pavlovian incentive learning.

It is important to point out that these possibilities are not mutually exclusive; therefore, the fact that impairment of DA transmission can be unequivocally demonstrated to result in one of the above effects cannot be taken as evidence to negate the occurrence of the others. Since it is not unlikely that the above mechanisms are all operative in complex paradigms of instrumental responding, the analysis of each one of these components has relied on the use of simple behavioral paradigms designed to specifically estimate a given component or on parametric studies of complex instrumental paradigms that distinguish the contribution of each component. An additional approach to circumvent the difficulties of the multicomponent nature of the effect of DA receptor blockade on behavior has been that of manipulating DA transmission in circumscribed brain regions, on the assumption of a differential localization of the different substrates of the action of these drugs on behavior.

5.1.1. Testing the original anhedonia hypothesis

The original anhedonia hypothesis has been tested in studies of the effects of DA receptor antagonists and 6-OHDA lesions of DA neurons on operant and free-feeding measures of sucrose or saccharin reward. However, in spite of the large number of studies performed (reviewed by Di Chiara, 2000) the results obtained are compatible with any of the possibilities indicated above and therefore do not allow to be distinguished.

Taste reactivity (Grill and Norgren, 1978) has been proposed as a means to investigate the hedonic properties of taste stimuli (Berridge, 2000). In taste reactivity tests, solutions are infused directly into the mouth of the subject through intraoral cannulae. Sweet solutions evoke the emission of a characteristic pattern of hedonic reactions (frontal tongue protrusion; lateral tongue protrusion, paw licking) while bitter solutions evoke aversive reactions (gapes, forelimb flails, head shakes) (Grill and Norgren, 1978). These reactions are affected by drive state and by drugs and brain lesions in a manner compatible with the notion that they reflect the motivational valence and value of the taste stimulus. Using this paradigm, Treit and Berridge (1990) showed that administration of a large dose of haloperidol (1 mg/kg) fails to induce changes in hedonic or aversive taste reactions. No change in hedonic taste reactivity was also obtained after 6-OHDA lesions by Berridge et al. (1989) and by Berridge and Robinson (1998). Leeb et al. (1991) however reported that pimozide reduces hedonic reactions to sucrose; moreover, Parker and Lopez, Jr. (1990) reported that pimozide enhances the aversiveness of quinine solutions. In order to investigate the reason for these discrepancies, Berridge and Parker and their collaborators joined together in a collaborative study (Pecina et al., 1997) and reached the following conclusions: pimozide reduces hedonic taste reactions to sucrose, but this effect takes place slowly and, in any case, only after the first minute of the trial, in agreement with Treit and Berridge (1990) who utilized 1 min infusions. Because of this, and also since aversive reactions to quinine were found to be reduced, the authors attributed the effect of pimozide to a sensorimotor impairment rather than to a blunting of taste hedonia.

Results inconsitent with the anhedonia hypothesis have also been obtained in studies of sucrose intake and preference in mice carrying a deletion of tyrosine hydroxylase that results in brain DA levels as low as 1% of those of the wild strain (Cannon and Palmiter, 2003). These mice are aphagic and akinetic but show a preference ratio of sucrose and saccharin over water similar to the wild-type mice. Moreover, when shifted from water-drinking to sucrose, dopamine deficient mice show heightened changes in the microstructure of drinking (increase in bout duration, increase of licks per bout, increase in the lick rate) typical of a shift to a higher value reward (Cannon and Palmiter, 2003). Nonetheless, dopamine deficient mice showed a decrease in total bouts and licks, most likely as a result of a performance deficit. These observations are reminiscent of earlier studies comparing sucrose ingestive behavior from drinking tubes as compared to intraoral cannulae in normal adult rats and in rat pups. Thus, raclopride, while reducing the sucrose intake from drinking tubes in adult rats (Schneider et al., 1990) and from tissue on the bottom of a beaker in rat pups (Tyrka et al., 1992), failed to decrease the intake of sucrose infused intraorally through cannulae both in rat pups (Tyrka et al., 1992) and in adult rats (Tyrka and Smith, 1993). Similar observations were made with SCH 23390 in rat pups of 7 and 14 days (Tyrka and Smith, 1991). In adults, SCH 23390 reduced intake of intraoral sucrose only at doses much higher (~10 times) than those that inhibit intake by drinking from tubes. These observations can be explained if one assumes that sucrose intake in consumption tests, such as licking from the floor in pups or drinking

from tubes, involves two phases, an appetitive/preparatory phase which consists of approach by the subject the source of sucrose thus leading to contact of the mouth with the sweet source, tongue protrusion and licking, with consequent stimulation of gustatory receptors by the sweet taste. Once this is accomplished, the consummatory phase, related to the rewarding value of the taste and characterized by a rigid, almost stereotyped sequence of licking and swallowing, is initiated and carried on until it is progressively reduced and terminated by satiety. Impairment of DA transmission impairs the first appetitive/preparatory phase but not the second, purely consummatory one. Therefore, impairment of DA transmission impairs sweet reward by blunting the appetitive, approach phase of sweet reward but not its consummatory phase, related to the hedonic impact of the reward.

Evidence consistent with this conclusion has been recently obtained by Pecina et al. (2003) in the genetically-engineered DAT knockdown mice, who carry a subtotal reduction in the expression of DAT which results in an increased steady-state level of extracellular DA (Pecina et al., 2003). Compared to the wild-type mice, the knockdown mice show faster running for food in a straight runway and an increased food intake, which results in an increased body weight. This increased motivation for food was not the result of increased rewarding properties of food as estimated from the hedonic reactions to intraoral infusion of sucrose. These studies therefore are consistent with the idea that sweet reward is independent from DA and that DA plays a role in the incentive, rather than the rewarding properties of food.

From an entirely different approach, studies by Salamone and coworkers (Salamone, 1992, 1994; Salamone et al., 1997, 1999, 2003; Salamone and Correa, 2002) provide further evidence against a role of DA in the hedonic properties of food. Thus, in rats allowed to choose between an operant response and a simple approach response to obtain food, impairment of DA transmission by systemic DA-receptor antagonists increases food consumption by direct approach while reducing operant responding for food. Therefore, in concurrent choice tasks, DA receptor blockade cause subjects to reallocate their choices in the direction of behaviors that involve less effort (Salamone et al., 1997, 1999, 2003; Salamone and Correa, 2002). Local infusion of DA receptor antagonists and lesion of NAc DA by local infusion of 6-OHDA produce effects in concurrent choice tasks that closely resemble those observed after systemic neuroleptics. Moreover, the effects of accumbens DA lesions on operant responding for food can vary greatly depending upon the task. For example, some schedules of reinforcement (e.g. FR1) were insensitive to the effects of DA lesions, whereas others were highly sensitive (> Fr50). Accumbens DA lesions slow the rate of operant responding, blunt the rate-facilitating effects of moderate-sized ratios, and enhance the rate-suppressing effects of very large ratios (Salamone et al., 1997, 1999; Aberman and Salamone, 1999). These observations, while inconsistent with a role of DA in the hedonic impact of food reward, are compatible with an energizing function of DA, not dissimilar from an incentive arousal role. Specifically, accumbens DA, rather than mediating the rewarding impact of food, would be important for overcoming behavioral constraints, such as work-related response costs, and for enabling to engage in vigorous responses, such as barrier-climbing, or to emit large numbers of responses in ratio schedules in the absence of primary reinforcement (Salamone et al., 1997, 1999, 2003).

Additional evidence against a simple hedonic function of the NAc DA transmission in food reward is provided by the observation that the behavioral effects of impairment of DA transmission on a concurrent operant and approach response for food are different

from those of extinction or outcome devaluation by prefeeding or by administration of anorectic drugs (Salamone and Correa, 2002; Salamone et al., 2003).

Finally, intraaccumbens infusion of doses of D1 and D2 DA receptor antagonists that impair locomotion and rearing do not impair food intake and latency to feeding (Baldo et al., 2002).

5.1.2. The role of performance impairment

A major difficulty with a role of DA in reward derives from the inextricable relationship between the response-reinforcement construct and the motor performance and by the fact that DA plays an important role in motor functions. Indeed, the possibility of a performance effect although 'subtle' and peculiar, is considered even by Salamone (1992) as an interpretative framework of the effect of impairment of DA transmission on responding.

An attempt to distinguish performance from reinforcement/motivational effects is provided by matching law studies. In these studies, the effect of the DA receptor blockers on the relation between the response rate and the reinforcement rate in concurrent, alternate or sequential interval (VI or RI) schedules of operant responding is investigated (de Villiers and Herrnstein, 1976). It has been observed that DA receptor blockers not only reduce maximal response rate (Ks) (an index of performance impairment) from the beginning of the session but also increase reinforcement needed for maintaining half-maximal response rate (Kh) (an index of reduced reinforcement impact/motivational strength) late in the session (Willner et al., 1990; Phillips et al., 1991a). In the case of SCH 23390 and sulpiride, reduction in the motivational strength takes place late in the session at doses that do not affect performance early in the session (Phillips et al., 1991b). This observation is particularly relevant, since SCH 23390 and sulpiride have been reported to be unable to elicit within-session reductions of responding in conventional operant schedules (Sanger, 1987; Sanger and Perrault, 1995). In view of this, the observation that atypical neuroleptics fail to induce within-session reduction of responding on conventional schedules (Sanger and Perrault, 1995) does not exclude that they induce a within-session reduction on multiple schedules.

5.1.3. Testing the effect of DA receptor blockers in their absence

One way to overcome the confounding influence of performance impairment in studies of the effect of DA receptor blockers on motivated behavior consists in testing for the action of these drugs in their absence (Beninger, 1989; Ettenberg, 1989).

In a first series of studies haloperidol (0.075 and 0.15 mg/kg) was given intermittently on 10 (33%) out of 30 single daily sessions of running for food or water reinforcement in a straight runway (Ettenberg and Camp, 1986a,b). During the following 12 days the responding was tested in single daily sessions under extinction conditions. No impairment in movement initiation or in performance was observed under haloperidol, as indicated by the unchanged latency to leave the start box and the marginal increase in the time to reach the goal box. On the extinction phase, rats intermittently exposed to haloperidol showed a significant resistance to extinction compared to controls not given the drug; this effect, in turn, was similar to that observed in a group in which reinforcement was omitted on the same proportion of trials (33%). Thus, haloperidol did not impair the maze-running performance for food or water reward but slowed down the rate of extinction of the

motivated response on subsequent drug-free test sessions much in the same way as intermittent nonreinforcement. Failure of haloperidol to affect maze-running on trial suggests that at the doses given, haloperidol differentially affects the expression of the response-eliciting (incentive) properties of conditional stimuli predictive of reward and the reinforcing properties of reward: while the first ones are intact, the second are impaired.

These results have been confirmed by Feldon et al. (1988) who also tested the effect of haloperidol given daily during reinforced and nonreinforced sessions of a partial reinforcement paradigm (50% of the responses unrewarded). Under these conditions, haloperidol, contrary to the predictions of the anhedonia hypothesis, did not facilitate extinction. Further studies by Feldon and Weiner (1991), performed on the multiple daily sessions, show that, contrary to the observations in the single daily sessions, haloperidol fails to impair reinforcement as indicated by failure to produce a resistance to extinction, when given during continuous reinforcement and a facilitation of extinction, when given during partial reinforcement. These observations, coupled with the fact that haloperidol increases the rate of extinction when given during extinction, have been taken to indicate that haloperidol reduces the impact of reinforcement only on single daily reinforcement schedules while it increases the impact of nonreinforcement both on single and multiple daily reinforcement schedules (Feldon and Weiner, 1991). The difference between the impact of haloperidol on reinforcement in single versus multiple schedules has been explained by the different learning processes operative in the two conditions (Feldon and Weiner, 1991). Thus, while responding on the multiple daily trial schedules utilizes the response–outcome (instrumental) relationships, this is not the case in single trial sessions, which depend on the acquisition of incentive properties by stimuli that precede responding. Therefore, in single schedules, neuroleptics might reduce the impact of reinforcement by impairing incentive learning.

In a further series of studies by Ettenberg and associates, stimuli were explicitly paired (CS+) or unpaired (CS−) with reinforcement, thus becoming predictive of reinforcement and, respectively, of nonreinforcement in a straight runway. Haloperidol (0.15–0.30 mg/kg) failed to increase run times in response to the CS whereas it strongly increased in a drug-free test, performed on the next day. Similar results were obtained with conventional reinforcers, such as food (Horvitz and Ettenberg, 1991) and sex (Lopez and Ettenberg, 2001) and drug reinforcers (i.v. heroin) (McFarland and Ettenberg, 1995). Results consistent with an impairment of reinforcement independently from motor impairment have been obtained by the same group on the response-reinstating properties of reinforcement by conventional and drug reinforcers. In this paradigm, subjects are first trained to run the maze in response to reward (food, Horvitz and Ettenberg, 1988; water, Ettenberg and Horvitz, 1990) or to drug reward (i.v. amphetamine, Ettenberg, 1990; i.v. heroin, Ettenberg et al., 1996). Once the response is extinguished by a series of nonreinforced sessions, responding is reinstated by a single reexposure to the reward in the goal box after haloperidol or saline administration. On the next day, testing for maze-running in the absence of haloperidol showed a reduction of response in the haloperidol, as compared to the saline-exposed group.

These observations could be explained either by an impairment of instrumental response–reinforcement (haloperidol impairs the ability of the reinforcer to strengthen extinguished act–outcome relationships or S–R associations) or of Pavlovian stimulus–reinforcement (haloperidol impairs the ability of the reinforcer to strengthen the incentive properties of the goal box).

These studies, however, have been performed in a straight runway and the response measured (run time to the goal box) is a natural and elementary incentive response as approach behavior. This response may not be equivalent to an unnatural and complex response such as bar-pressing. Because of this, some authors do not regard the maze-running paradigms as an expression of instrumental behavior, but instead, of the Pavlovian and incentive–motivational responding, being based on learning of stimulus-contingencies, rather than response-contingencies (Dickinson and Balleine, 1994). Therefore, the apparent similarity between the two effects of neuroleptics, the within-session impairment of bar-pressing shown by Wise and colleagues and the delayed reduction of maze-running shown by Ettenberg and colleagues, may not be a reflection of their homology but rather of their analogy, that is, of a commonality in a phenomenological aspect rather than in a basic one. Thus, although for the principle of parsimony, one would favor a unitary mechanism of the effect of neuroleptics in operant responding and in the maze-running paradigms, the differences inherent to them make this principle not readly applicable to this specific case.

An additional reason for considering the impairment induced by neuroleptics on reinforcement by bar-pressing as not homologous to that obtained on reinforcement by maze-running is the fact that while D1 and D2 receptor antagonists are similarly effective in producing within-session reduction of bar-pressing, only D2 antagonists impair reinforcement in maze-running paradigms (Chausmer and Ettenberg, 1997). This difference is particularly puzzling given the circumstance that D1 receptor antagonists have been indicated to be more specific than D2 antagonists in reducing reinforcement as compared to their ability to impair performance as estimated from their ability to induce microcatalepsy (Fowler and Liou, 1994, 1998) and to modify the reward summation function for ICSS (Hunt and Atrens, 1992). Failure of D1 receptor blockade to impair reinforcement in the paradigm of Ettenberg et al., however, is also inconsistent with the idea that this paradigm involves Pavlovian stimulus reinforcement rather than response reinforcement and that D2 antagonists given on trial act on the acquisition of stimulus-reward association.

Notwithstanding the above caveats, we favor the interpretation of the effects of neuroleptics in the paradigm of Ettenberg and colleagues as due to an impairment of Pavlovian incentive learning rather than of response reinforcement. These studies also indicate that, once acquired by Pavlovian learning, the expression of the incentive properties of stimuli are resistant to neuroleptics.

5.2. DOPAMINE AND INCENTIVE-MOTIVATION

As already referred, the observations by Gray and Wise (1980) and Phillips and Fibiger (1979) led Wise (1982) to a major revision of the original anhedonia hypothesis. This revised anhedonia hypothesis took from the incentive-motivational theories of Bindra (1974, 1978) the notion that incentives acquire not only the response-eliciting properties but also the hedonic properties of the reward to which they have been conditioned. The revised anhedonia hypothesis has many homologies with another influential hypothesis of the function of DA, that of the role of DA in the transduction of motivation and action (Mogenson et al., 1980). This hypothesis, however, was originally referred to the ventral striatum as an interface between motivation and action. Other authors have favored the idea of a response-energizing (Salamone, 1987, 1997) and a preparatory role of DA (Blackburn et al., 1987, 1992). The response-energizing hypothesis has already been

discussed. The preparatory hypothesis comes from the observation that pimozide reduces preparatory responses (the number of entries into a niche where food is expected) at doses that do not reduce food consumption when the food itself is available (Blackburn et al., 1987, 1992).

Both the response-energizing and the preparatory roles of DA can be regarded as aspects of an incentive view of the function of DA. Thus, activation of DA transmission by incentive stimuli predictive of reward availability might facilitate the sustained emission of instrumental responses and of appetitive behaviors of search and approach.

Other hypotheses related to a motivation-to-action view of the role of DA are those that envision a gain-amplifying (Robbins et al., 1989) and a response-switching role of DA (Cools, 1980; Weiner, 1990; Redgrave et al., 1999; Mehta et al., 2004).

Berridge (1996) and Berridge and Robinson (1998) have proposed a distinction between hedonic properties (liking) and response-eliciting properties (wanting) of incentive stimuli and have assigned to DA a role in response-eliciting but not in hedonic properties. This hypothesis is based on the observation that lesions of DA neurons and pharmacological blockade of DA transmission fail to impair the behavioral hedonic reactions to highly palatable tastes, such as those of sucrose solutions infused intraorally (Berridge, 1996; Berridge and Robinson, 1998). However, one could argue that in these studies, the experimental approach (taste reactivity scores) utilized for testing the anhedonia hypothesis is not appropriate to the kind of hedonia to which the anhedonia hypothesis refers. Indeed, Wise himself (1982) originally indicated in the ability of DA-receptor blockers to prevent amphetamine-induced euphoria a test of the anhedonia hypothesis. Therefore, taste hedonia, being a form of stimulus-bound hedonia, is not appropriate to test the anhedonia hypothesis. That the drug-induced euphoria rather than the taste-hedonia is the true correlate of DA-dependent hedonia is suggested by the circumstance that in humans amphetamine-induced euphoria is correlated with drug-induced stimulation of DA transmission in the ventral striatum (Drevets et al., 2001). Moreover, a role of DA in euphoria is the tenet of current hypotheses on the role of DA in normal and abnormal mood states (eutimia, dystimia, depression, mania) (Papp et al., 1991). These observations are consistent with the notion of different kinds of hedonia and of a DA-independent stimulus-hedonia (taste hedonia) distinct from a DA-dependent state-hedonia (euphoria).

5.2.1. Dopamine and the expression of incentive-motivation

DA has been implicated in the expression as well as in the acquisition of incentive-motivation. Various hypotheses, since the revised anhedonia hypothesis, assume that DA mediates or modulates the expression of the incentive properties of stimuli. However, apart from their common 'incentive' label, these hypotheses differ substantially in some aspects critical for their testing and for their 'working'character. Thus, the term 'incentive' has been utilized in two different senses: a 'specific sense', referring to the directional response-eliciting properties of stimuli and in a 'nonspecific sense', referring to their generic response-arousing properties. We indicate the first as stimulus-bound incentive and the second as incentive arousal.

According to Berridge (1996), Berridge and Robinson (1998) and Robinson and Berridge (1993), DA is involved in a mechanism of 'incentive salience attribution'. By this mechanism, stimuli conditioned to a reward by DA-independent Pavlovian learning are imbued with response-eliciting (incentive) properties as a result of their conditioned

DA releasing properties. In relation to this, Berridge and Robinson (1998) explicitly hypothesize that an incentive stimulus derives its ability to elicit a response from the property, typical of reward-predictive stimuli (Schultz, 1998) of triggering a burst of spikes in DA neurons and a consequent phasic release of DA in the striatum. This assumption not only provides a mechanism for the role of DA in incentive responding but also suggests that in the Berridge and Robinson (1998) hypothesis stimulus-bound release of DA is envisioned to *enable* the response-eliciting properties of the stimulus that triggered it. This point is a critical one as it views the role of phasic DA in response expression in series between the stimulus and the response (stimulus-bound role).

5.2.1.1. Temporal properties of postsynaptic responses to DA: impact on incentive theories of DA function

The idea that the attribution of incentive salience is the result of a phasic stimulus-bound activity of DA neurons temporally placed in series between the stimulus that triggered it and the response is in contrast with the available evidence on the time-relationship between stimulus-bound burst activity in DA neurons and movement-related activity in basal ganglia output neurons. According to Schultz (1998), DA neurons fire with a delay of about 100 ms after the unpredicted presentation of a reward or a reward-conditioned stimulus. On the other hand, Gonon (1997) has shown that stimulation of the medial forebrain bundle by four 15 Hz pulses results in a short latency (< 20 ms) spike following each pulse and a delayed excitation in postsynaptic striatal neurons that starts about 200 ms after the beginning of the stimulation and lasts for as long as 1 s! The late excitation but not the early spike was sensitive to D1 blockade with SCH 23390. On the other hand, in vivo studies in the rat and in the monkey have shown that it takes less than150 ms for a behaviorally significant stimulus to produce a response in the efferent basal ganglia neurons of the substantia nigra pars reticulata and of the medial pallidal segment (Hikosaka and Wurtz, 1983). Therefore, by the time the presentation of a stimulus results in activation of DA neurons (100 ms) and DA starts to elicit its post synaptic effects (>200 ms), responsive units along the efferent pathway of the basal ganglia would have already initiated their discharge sequence that leads to the inhibition of output neurons in the SN and GP by fast GABA receptors. Thus, by the time stimulus-bound activity of DA neurons takes place, transfer of the stimulus beyond the DA synapse and down the basal ganglia output has already taken place. These observations make it unlikely that, as proposed by Berridge and Robinson (1998), phasic DA transmission is online with action. A role of DA on the impact of stimuli that follow the one that triggered it is also a tenet of the proposal by Schultz (1998) that phasic DA release is a teaching signal, strengthening future transmission through striatal synapses activated in coincidence with it. Redgrave et al. (1999) have argued that the latency of the DA reward signal is too short for this signal to be able to teach anything significant about the reward itself and even less so for teaching anything about the motor response to the reward. According to Redgrave et al. (1999), the latency of the DA signal is between 50 and 100 ms from a triggering visual stimulus while the latency of a saccade is between 80 and 100 ms for an express saccade to as long as 180–200 ms for a regular saccade. Apart from the circumstance that an unpredicted highly salient stimulus, such as the one predicting reward, is worth an express rather than a regular saccade (thus making the reward DA signal already late in respect to the action it should be a determinant of), what Redgrave et al. (1999) have not accounted for in their calculations is the delay, intrinsic to the

metabotropic/modulatory nature of the DA cellular action of DA. Thus, as pointed out above, if one takes into account these figures, the temporal delay of the action of DA (>200 ms) is such as to exceed even the latency of a regular saccade.

A direct relationship between the release of DA and action is also incompatible with the circumstance that stimuli effective in activating DA neurons are not necessarily action-triggers but might rather serve as instruction signals predictive of action-triggering stimuli that do not necessarily stimulate DA neurons; eventually, activation of DA neurons rather than preceding, can actually follow responding, being elicited by response-outcome (reward) (Schultz, 1998). Therefore, if indeed release of DA plays a role in the expression of incentive responding this role cannot be envisioned to act on responding to stimuli that promoted the release of DA, but rather on those that followed it.

5.2.1.2. Experimental evidence against a stimulus-bound incentive role of DA

Experimental studies do not support a stimulus-bound role of DA in the response-eliciting properties of conditioned incentives. Thus, presentation of a novel CS reinstates responding for ICSS blocked by pimozide (Franklin and Mc Coy, 1979), an observation that contrasts with the idea that neuroleptics specifically impair the incentive effects of stimuli. Studies by Ettenberg and associates show that neuroleptics do not impair incentive responses to CS (Horvitz and Ettenberg, 1988, 1991; McFarland and Ettenberg, 1995, 1999). Particularly relevant to this issue is the finding that in rats trained to run a straight maze in response to discriminative olfactory cues predictive of the occurrence (S+) or absence (S−) of food or of i.v. heroin reward in the goal box, haloperidol (0.075–0.30 mg/kg) failed to increase the maze run times in response to the CS+. Haloperidol (0.15–0.30 mg/kg) also failed to affect preference for the CS+ over the CS− (McFarland and Ettenberg, 1995, 1999). Therefore, haloperidol, at doses that impair reinforcement, did not affect the activational (CS-induced maze-running) nor the directional/discriminative properties of a discrete CS. The same doses of haloperidol, however, increase run time of sexually naive male rats in response to oestrus female cues (Lopez and Ettenberg, 2001). Moreover, the same or even lower doses of the haloperidol prevented the ejaculation-induced decrease in runtime in response to oestrus and nonoestrus female cues (Lopez and Ettenberg, 2000). Therefore, according to Ettenberg and colleagues, DA receptor blockade while not impairing the consummatory aspect of sexual reward (copulation and ejaculation), impairs sexual reinforcement (i.e. the ability of sexual reward to strengthen incentive responses upon which it is contingent) as well as approach responses to primary, unconditioned sexual incentives (olfactory sexual stimuli) and to food or drug-conditioned incentives. Thus, the observations of Ettenberg and colleagues challenge the report by Blackburn et al. (1987) that pimozide specifically reduces incentive/preparatory responses (visits to the niche where food is expected) in response to a food-predictive CS. McFarland and Ettenberg (1999) attribute this discrepancy to failure of Blackburn et al. (1987) to take into account the effect of pimozide on basal responding in the absence of the CS+.

5.2.1.3. Incentive arousal role of dopamine

The above observations, while indicating that DA may not be essential in general for Pavlovian responding to discrete incentive stimuli (stimulus-bound incentive responding), leave open the possibility that DA plays a role in the facilitation of responding associated

with states of behavioral arousal induced by reinforcers. We will refer to this state as 'incentive (motivational) arousal'. Such a state is thought to strongly facilitate instrumental responding for the reward to which the incentive has been conditioned as well as for other rewards. This property is in essence that of nonspecific transfer from Pavlovian to instrumental (PIT). Consistent with the notion of a role of DA in incentive arousal, amphetamine facilitates PIT (Wyvell and Berridge, 2000). Conversely, DA receptor blockers impair the reinstatement of instrumental responses for drug self-administration induced by drug cues and contexts. Thus, the D2 antagonist nafadotride and two D1 antagonists, SCH 23390 and SKF 38393, impaired reinstatement of operant responding by discriminative cues after extinction of cocaine self-administration (Ciccocioppo et al., 2001; Weiss et al., 2001). Systemic SCH 23390 and raclopride also impaired reinstatement of operant responding upon reexposure to the same contextual cues, where cocaine self-administration had taken place (Crombag et al., 2002). A DA-dependent incentive arousal role of Pavlovian cues and contexts might be operative in the effects of neuroleptics and of 6-OHDA lesions of the NAc on instrumental responding by Salamone and colleagues. Thus, impairment of DA transmission slows the rate of operant responding, blunts the rate-facilitating effects of moderate size ratios and enhances the rate-suppressing effects of large ratios. Morover, in rats allowed to choose between an operant response and a simple approach response to obtain food, impairment of DA transmission increases food consumption by direct approach while reducing operant responding for food (Salamone, 1992, 1994; Salamone et al., 1997, 1999, 2003; Salamone and Correa, 2002). These results, on the other hand, are consitent with those of Ettenberg and colleagues that DA is not essential for simple incentive approach responses, such as maze-running (Horvitz and Ettenberg, 1988, 1991; McFarland and Ettenberg, 1995, 1999) and reinforce our suggestion of an incentive arousing rather than a stimulus-bound incentive role of DA.

DA might play this nonspecific incentive arousal role in relation to the experimental conditions of specific behavioral paradigms. One such condition might be that of schedule-induced adjunctive behavior. In this paradigm, cumulative arousal (Killeen et al., 1978) related to expectancy of a food pellet, insufficient per se to reduce food drive induced by an intermittent (1–4 min) schedule of food presentation, results in a steady increase of DA throughout the whole striatum (Church et al., 1987; McCullough and Salamone, 1992). This tonic increase of DA transmission might be instrumental for adjunctive behavior to take place. A similar mechanism might be operative in instrumental schedules. In both these conditions, build up of DA in the extracellular fluid would induce a state of incentive arousal that sustains and energizes responding. Under the CRF schedules, blockade of DA transmission impairs responding only after some delay, consistent with the within-session effect of neuroleptics on instrumental behavior (Wise, 1982).

According to this hypothesis, DA would be the substrate of an arousal state (incentive arousal) that nonspecifically increases the ability of incentives to faciltate instrumental responding. We speculate that motivational stimuli have DA-independent incentive properties whose ability in facilitating instrumental responding is amplified as a result of heightened DA transmission.

The notion of 'incentive arousal' described here is similar to that of the 'incentive state' of some early incentive theorists, particularly Cofer (1972) and Killeen (1975) and corresponds to the classic notion of behavioral arousing as distinct from directional effects of reinforcers. An incentive role of reinforcers related to their behavioral arousing influences was assumed to be the mechanism by which stimuli exert Pavlovian

influences on instrumental responding in the two-process theory of Rescorla and Solomon (1967).

The notion of an incentive arousal role of DA has some similarities with that envisioned by Wise (1982) in his revised anhedonia hypothesis. It is notable, however, that, even in the revised anhedonia hypothesis, the main function of DA remains that of mediating hedonia, consistently with the notion that incentives acquire not only the response-eliciting but also the hedonic properties of the rewards to which they are conditioned, thus becoming conditioned rewards (Bindra, 1974, 1978).

Incentive arousal role of dopamine and the behavioral effects of psychostimulants. An incentive arousal role of DA is best suited to explain many behavioral properties of psychostimulants. Indeed, the notion of an incentive role of endogenous DA is largely derived from the role attributed to DA as the substrate of the effect of psychostimulants on reinforcement and instrumental responding (Di Chiara, 1995). Psychostimulants elicit typical unconditional incentive effects in the form of approach towards stimuli and exploratory behavior related to novelty of the context. Psychostimulants also facilitate conditioned reinforcement (the ability of a Pavlovian CS to elicit responding instrumental to its presentation) (Robbins et al., 1989), an effect involving preliminary Pavlovian association with a reward and therefore related to the incentive properties of the stimulus.

Forward locomotion. Forward locomotion and exploratory behavior constitute the typical unconditioned incentive response of rodents to a novel environment (Bardo et al., 1996). This response is impaired by blockade of DA transmission. This observation is consistent with the differential effect of DA receptor blockade on simple approach responses elicited by conditioned vs. unconditioned incentives (Lopez and Ettenberg, 2000). Drugs of abuse induce forward locomotion and patterns of exploratory behavior that mimic to a certain extent the behavioral response to a novel environment. This property applies not only to psychostimulants and nicotine but also to drugs with depressant properties, such as narcotic analgesics and ethanol, at least within an appropriate dose range and time after administration. The evidence supporting this conclusion is both experimental and correlative. Blockade of DA receptors by drugs acting on D1 or D2 receptors, impairment of vesicular storage of DA by reserpine or blockade of DA synthesis by α-methyl-p-tyrosine impair the locomotor stimulant effects of psychostimulants, ethanol and nicotine given at low doses that are also the threshold for stimulation of DA transmission in the nucleus accumbens (see reviews by Beninger, 1983; Wise and Bozarth, 1987; Stolerman and Shoaib, 1991; Di Chiara and North, 1992; Di Chiara, 1995). The role of DA in the hypermotility elicited by systemically administered opiates has been debated for some time although the weight of evidence strongly indicates that in intact animals systemic opiates elicit hypermotility by a DA-dependent mechanism (see discussion in Di Chiara, 1995).

The importance of ventral striatal DA in the locomotor response to psychostimulants was demonstrated by current classic studies showing that manipulating ventral striatal DA transmission by lesion (Kelly and Iversen, 1976) or local infusion of DA receptor antagonists impairs while local infusion of DA receptor agonists (Pijenburg et al., 1976) evokes forward locomotion and exploratory behavior in a novel environment. Subsequent studies have attempted to establish which one among the main subdivisions of the ventral striatum (NAc shell, NAc core, olfactory tubercle) could be responsible for this function. This issue, however, is highly controversial.

Electrolytic lesions restricted to the NAc *shell* were reported by Weiner et al. (1996) to potentiate the locomotor effects of systemic amphetamine without affecting spontaneous activity; NAc *core* lesions did not modify spontaneous or amphetamine-induced locomotion (Weiner et al., 1996). Different results were reported by Parkinson et al. (1999) after excitotoxic lesions; thus, lesions of the NAc *shell* induced hypomotility and reduced the locomotor effects of amphetamine while NAc *core* lesions induced hypermotility and potentiated amphetamine hypermotility. Jongen-Relo et al. (2002) observed a slight increase of spontaneous locomotion after excitotoxic lesions of the *shell* and no effect after *core* lesions, but the response to amphetamine was not tested. The observations of Parkinson et al. (1999) in turn agree with those of Maldonado-Irizarry and Kelley (1995) showing that excitotoxic NAc *core* lesions enhanced spontaneous locomotion while *shell* lesions were without effect. After 6-OHDA lesions, on the other hand, the degree of reduction of locomotion in response to amphetamine was a direct function of the loss of DA terminals in the NAc *core* and an inverse function of the loss in the NAc *shell* (Boye et al., 2001; Sellings and Clarke, 2003). Studies with local infusion of DA receptor antagonists do not distinguish between *shell* and *core* since both D1 and D2 antagonists impair spontaneous locomotion in a free feeding paradigm to a similar extent from the shell and from the core (Baldo et al., 2002), the only difference being a tendency to a greater inhibition by intrashell infusion of D1 antagonists. Intracerebral amphetamine was similarly effective in evoking locomotion from the *shell* and from the *core* in a number of studies (Ikemoto, 2002; Johnson et al., 1996). West et al. (1999), instead, observed a higher locomotor effect by intracore than intrashell amphetamine in a rat strain selected for vigorous swimming activity. However, clearcut differences between shell and core were observed in outbred rats in the study of Swanson et al. (1997) after local dopamine, SKF 82958, quinpirole and mixtures of these two agonists and in those of Choi et al. (2000) and of Ikemoto (2002) after quinpirole-SKF 38393 mixtures. In these studies utilizing direct agonists hypermotility was evoked specifically from the *shell* and this effect was particularly clear after infusion of D1 agonists, alone or in combination with quinpirole.

Taken as a whole, the available evidence seems to point to the medial NAc shell as the most sensitive site for evoking the forward locomotion by DA agonists in the rat.

Facilitation of conditioned reinforcement and transfer from Pavlovian to instrumental. Facilitation of conditioned reinforcement by amphetamine and transfer from Pavlovian to instrumental (PIT) are probably the best models of the incentive-arousing function of DA. Stimulation of DA transmission in the nucleus accumbens facilitates the expression of secondary (conditioned) reinforcement (Hill, 1970; Robbins, 1975; Beninger et al., 1991). In these studies, conditioning of an otherwise neutral stimulus by repeated association with a primary stimulus is first established; the effect of stimulation of DA transmission on the ability of the secondary stimulus to act as a reinforcer in the acquisition of a new operant task is then tested.

Amphetamine given systemically or infused in the nucleus accumbens facilitates responding with conditioned reinforcement and this effect can be prevented by doses of neuroleptics or by 6-OHDA lesions that per se do not impair normal responding (Hill, 1970; Robbins, 1975; Beninger et al., 1983; Taylor and Robbins, 1984; Kelley and Delfs, 1991). This effect has been explained to be due to the positive motivational properties of amphetamine (Hill, 1970) or to its activational properties (Robbins et al., 1983).

The relevance of these observations for the role of DA in the behavioral properties of nonpsychostimulant drugs of abuse is unclear. Thus, systemic morphine and even systemic cocaine reportedly fail to increase the control over behavior by conditioned stimuli (Robbins et al., 1983).

Lesion studies have attempted to clarify the role of the two subdivisions of the NAc in the facilitation of conditioned reinforcement by amphetamine and in the expression of PIT. Excitotoxic lesions of the NAc shell impair the potentiation of responding with conditioned reinforcement induced by amphetamine (Parkinson et al., 1999). Also consistent with a role of the NAc shell in incentive arousal is the facilitation of transfer from Pavlovian to instrumental (PIT) responding induced by intra-NAc shell infusions of amphetamine (Wyvell and Berridge, 2000). However, contrasting results have been obtained by different laboratories on the effect of excitotoxic NAc shell versus core lesions on PIT. While Corbit et al. (2001) reported an impairment of PIT after NAc shell but not NAc core lesions, just the contrary, i.e. an impairment after NAc core but not NAc shell lesions, was reported by Hall et al. (2001). The reason for this discrepancy might be related to differences in behavioral paradigm. Thus it appears that lesions of the NAc core impair PIT in paradigms in which transfer is made dependent upon discrimination between active and inactive lever (specific PIT) while NAc shell lesions impair the activational aspects of PIT in terms of instrumental response rate without affecting its specificity (nonspecific PIT). This is consitent with the involvement of the NAc core in directional/discriminative aspects of instrumental responding related to its input from the anterior cingulate cortex and the basolateral amygdala, whose excitotoxic lesions reproduce the loss of discrimination between active and inactive lever in responding with secondary reinforcement observed after NAc core lesions. Finally, the NAc core more than the shell seems involved in the impairment of instrumental performance under schedules with high response ratios after 6-OHDA lesions of the NAc (Aberman and Salamone, 1999).

From the above analysis two views of the behavioral function of the NAc shell versus core emerge: according to one view (Corbit et al., 2001) the functions of the NAc core and of the NAc shell are distinct and pertain to separate, although interacting, aspects of responding, instrumental and Pavlovian, respectively. According to another view (Everitt et al., 1999), the NAc core plays a role in the influences exerted by Pavlovian stimuli on instrumental behavior as well as in directional/discriminative aspects of instrumental responding while the NAc shell is involved in the incentive influences exerted by unconditioned stimuli.

For what specifically concerns the role of NAc DA, we would propose a role of the NAc shell DA in incentive arousal, i.e. in the nonspecific amplification of instrumental responding by incentives and a role of NAc core DA in coupling the above influences to directional functions typical of this area. Therefore, a role of DA in response selection would not be carried by DA itself but would be the result of its modulatory influences on areas, such as the NAc core, that process information essential for directional aspects of responding.

Dopamine and reinstatement of responding by drug priming. Reinstatement of operant drug seeking by drug priming might be a further expression of the incentive arousal role of DA.

In operant reinstatement paradigms, animals are trained to self-administer a drug by pressing a lever for intravenous drug infusion. Once responding is acquired, the drug is

withdrawn and responding on the drug-active lever is extinguished. After extinction, the ability of acute exposure to drugs (priming) or nondrug stimuli to reinstate responding on the drug-lever is tested (Stretch et al., 1971; Stewart and de Wit, 1987). In these operant paradigms the effect of drug priming on responding is tested during the time-course of the drug itself and therefore one cannot avoid the confounding influence of performance effects exerted by experimental manipulations of drug priming. To circumvent this problem, the testing for reinstatement and the reversible manipulations of DA transmission are performed on the day after exposure to the drug, when the effect of the pharmacological manipulations of DA transmission are over. This feature, however, requires the use of paradigms based on Pavlovian (e.g. maze-running place preference) rather than operant responses (see Shalev et al., 2002 for review). Reinstatement of maze-running by priming with conventional (food, Horvitz and Ettenberg, 1988; water, Ettenberg and Horvitz, 1990) and drug reinforcers (i.v. amphetamine, Ettenberg, 1990; i.v. heroin, Ettenberg et al., 1996; McFarland and Ettenberg, 1997) is impaired by haloperidol given on trial (exposure to the primer) but not on test (expression of the incentive response). Apparently contrasting results have been obtained in operant paradigms: raclopride, a specific D2 antagonist, and flupentixol, a D1–D2 antagonist, impaired heroin-primed and the D1 antagonist SCH 23390 impaired cocaine-primed (Norman et al., 1999) and heroin-primed (Shaham and Stewart, 1996) reinstatement of operant responding. Thus, while DA receptor blockade impairs the expression of reinstatement in operant paradigms, it does not in Pavlovian paradigms.

The action of cocaine on reinstatement is mimicked by amphetamine, GBR 12909, a specific DA reuptake inhibitor, and by D2 agonists but not by D1 agonists (Shalev et al., 2002). Psychostimulant priming reinstates heroin seeking after prolonged (De Vries et al., 1998, 1999) but not short withdrawal periods (deWit and Stewart, 1983). Moreover, the D2 agonists bromocriptine (Wise et al., 1990) and quinpirole (DeVries et al., 1999, 2002) reinstate heroin seeking. Similar effects are obtained by intraaccumbens amphetamine (Stewart and Vezina, 1988) and intraVTA morphine (Stewart, 1984), the two manipulations that increase DA transmission in the NAc. These observations indicate that reinstatement by drug priming is the result of stimulation of NAc DA transmission not only in the case of psychostimulants like cocaine and amphetamine but also in nonpsychostimulants like heroin. While this is expected in the case of cocaine, it is in contrast with the results of self-administration studies in the case of heroin. This suggests that reinstatement is not directly related to the reinforcing properties of the drug as expressed in self-administration paradigms. The nonspecific properties of this effect are consistent with an incentive-arousing nature related to DA release in the NAc (Stewart et al., 1984) rather than with a relationship with the discriminative stimulus properties of the drug primer (Stolerman, 1992; Bergman and Katz, 1998). Studies on cocaine-primed reinstatement show that although DA infused in the NAc core reinstates extinguished responding for cocaine, and in spite of the fact that cocaine priming increases DA in the NAc, intraNAc core fluphenazine fails to impair reinstatement of cocaine responding (Cornish and Kalivas, 2000; McFarland and Kalivas, 2001). On this basis it has been concluded that an increase of DA in the NAc, although sufficient, is not necessary for cocaine-primed reinstatement (Cornish and Kalivas, 2000; McFarland and Kalivas, 2001). On the other hand, intraPFCX fluphenazine does prevent reinstatement (McFarland and Kalivas, 2001). Since increase of NAc DA is sufficient for reinstatement, the observation that reinstatement by systemic cocaine, which is known to increase DA in

the NAc, is dependent upon DA stimulation in the PFCX would mean that PFCX DA is a gate for impulses generated by stimulation of DA transmission in the NAc. On this basis it has been suggested that the stimulation of DA in the NAc secondarily activates DA transmission in the PFCX which in turn enables, through activation of a glutamatergic PFCX-NAc pathway, the expression of increased NAc DA transmission into reinstatement of responding for cocaine (McFarland and Kalivas, 2001). Consistent with this explanation is the observation that the blockade of AMPA-GLU receptors in the NAc core impairs reinstatement (McFarland and Kalivas, 2001). Therefore, activation of GLU transmission in the NAC core might play a permissive role for the behavioral expression of increased DA transmission in the NAc core. A distinctive feature of this hypothesis is the idea that reinstatement is the result of a combined activation of NAc and PFCX DA transmission. After systemic cocaine the increase in DA transmission would be secondary to a direct effect of cocaine in the PFCX while after intraNAc DA reinstatement would be the result of the behavioral activation induced by this manipulation. This hypothesis however does not account for the failure of intraNAc flupentixol to impair reinstatement and, vice versa, for the ability of intraPFCX DA alone to induce reinstatement. In fact, this manipulation is not expected to increase DA in the NAc, an effect that should be a prerequisite for the ability of cocaine to induce reinstatement. A solution to this puzzle is now provided by the recent finding that intraNAc shell but not intraNAc core infusion of SCH 23390 prevents reinstatement of responding for cocaine (Anderson et al., 2003). These observations indicate that the failure of flupenthixol to prevent reinstatement when infused in the core is not indicative of an independence of reinstatement from DA but of an inadequacy of that manipulation to show the role of NAc DA in this response. This important study also indicates that it is the DA of the NAc shell to provide the incentive for reinstatement, a notion fully consistent with the role here attributed to NAc shell DA as a substrate of incentive arousal.

5.2.2. Dopamine and the acquisition of incentive-motivation

An alternative interpretation of the role of DA in instrumental responding is one that still implicates incentive–motivation except that this role would not take place at the level of the expression of incentive influences but of their acquisition, i.e. on Pavlovian incentive learning. The ability of a stimulus, conditioned to a reward or punisher (US), to elicit a 'consummatory' (Konorsky, 1967) CR is not impaired by the administration of DA receptor blockers during CS-US pairing (Beninger, 1983). Large doses of chlorpromazine given during shock-tone pairing trials did not prevent the ability of the tone to elicit conditioned emotional aversive responses on a subsequent test (Beninger et al., 1980b). Similarly, pimozide failed to impair conditioned prod burying when administered during prod-shock pairings (Beninger et al., 1980a). Moreover, neuroleptics did not impair the acquisition of an operant discrimination (Tombaugh et al., 1980) and 6-OHDA lesions did not impair learning of brightness discrimination in an electrified U maze (Price and Fibiger, 1975). The acquisition of discrimination in an underwater Y-maze is impaired by the administration of spiroperidol and by 6-OHDA lesions (Ranje and Ungherstedt, 1977a,b) but this effect has been explained by performance impairment during the learning phase resulting in delay of stimulus-reward association (Beninger, 1983). Haloperidol and pimozide reduce classical conditioning of the rabbit nictitating membrane, but this effect has been explained by a reduction of CS salience rather than by an impairment of CS-US association (Hunt, 1956; Harvey and Gormezano, 1981).

These negative studies contrast with a number of other studies showing that DA receptor blockers impair the ability of stimuli to acquire secondary reinforcing properties and to exert incentive influences on instrumental behavior in drug-free tests if administered during CS-US pairings. The earliest report of these effects is from Beninger and Phillips (1980). In 1980, these authors first preexposed rats to a two-lever operant box, where depression of one lever produced a 3-s tone; then rats were conditioned, in the absence of the levers, to tone-food pairings and finally were tested for responding on the tone lever. Pimozide (0.5 or 1.0 mg/kg), was administered in conjunction with the Pavlovian conditioning session (tone-food pairings); in this way, the ability of DA receptor blockade to impair the acquisition of secondary reinforcing properties by the tone was tested in drug-free instrumental sessions (Beninger and Phillips, 1980). Conditioned reinforcement, indicated by an increase of responding for the tone in the test session compared with responding on preexposure sessions, was obtained in the group conditioned under saline or under 0.5 mg/kg pimozide but not under 1.0 mg/kg pimozide (Beninger and Phillips, 1980).

In a further study, Hoffman and Beninger (1985) addressed the specificity of the effect of pimozide on the acquisition of secondary reinforcement. Thus, it was hypothesized that the effect of pimozide was due to an action on the strength of conditioning. This issue, in turn, tapped into the role of a performance impairment on the efficiency of conditioning under pimozide. Thus, various doses of pimozide (0.5, 1.0, 2.0 and 4.0 mg/kg) were tested for their effects on 2 and 4 days conditioning. Groups of rats administered with pimozide 1 h after each conditioning session in their home cage were run to control for cumulative drug effects unrelated to an action on conditioning. The results showed a reciprocal interaction between duration of conditioning and dose of pimozide: the longer the conditioning, the higher the dose of pimozide needed to impair its efficiency.

The same approach utilized in the above studies was applied by Beninger and Phillips (1981) to study the role of DA in the acquisition of transfer of classical conditioning to an operant discrimination (facilitation of responding instrumental to the presentation of the US to which the CS has been conditioned earlier by Pavlovian association, PIT). In the transfer study by Beninger and Phillips (1981), differently from the previous study on the acquisition of conditioned reinforcement (Beninger and Phillips, 1980), operant boxes were equipped with only one lever. In these conditions, noncontingent presentation of the food conditioned tone increased the rate of acquisition of operant discrimination during the test, and this effect was significantly impaired in the group given pimozide during Pavlovian pairing. The effect was significant in the first three sessions, marginal in the second three sessions (5–7) and nonsignificant in the third three sessions (8–10). No differences in the latency to eat the pellets during tone-food pairing were observed. State-dependency was excluded on the basis of the observation of the previous study (Beninger and Phillips, 1980). These observations have been confirmed in a recent study by Dickinson et al. (2000) on the effect of pimozide (0.25 mg/kg) and cis-flupenthixol (0.5 mg/kg), given during Pavlovian pairing of a CS+ with food or sucrose, on the ability of the same CS+ to increase responding for the relative US over the rate obtained under presentation of a CS+. Both pimozide and cis-flupenthixol reduced transfer when given during the Pavlovian training. Neuroleptics did not affect the rate of magazine entries during conditioning, excluding a role of an impairment of conditioning due to a performance effect; however, neuroleptics reduced responding when given during the instrumental sessions. This effect, rather than to a state-dependent mechanism,

already excluded by Beninger and Phillips (1980) in studies of the effect of neuroleptics on the acquisition of secondary reinforcement, can be attributed to an impairment of performance. The conclusion of this series of studies is that impairment of DA transmission by neuroleptics during Pavlovian conditioning of an arbitrary stimulus impairs the incentive effects of the stimulus on instrumental responding and its ability to acquire conditional reinforcing properties.

Impairment of Pavlovian incentive learning also provides an explanation for the observations of Ettenberg and collaborators.

5.2.2.1. Place-conditioning by conventional rewards: evidence for a role of dopamine in Pavlovian incentive learning

Further evidence for a role of DA in Pavlovian incentive learning comes from place-conditioning studies. This paradigm involves pairing of a specific context with a reward or a punisher (US) and testing the appetitive or aversive properties of the place (CS) under extinction (Carr et al., 1989; Hoffmann, 1989; Calcagnetti and Schechter, 1994; Tzschentke, 1998). As pairing is not contingent upon a response, this learning is Pavlovian in nature; however, the CR is, unlike the response to the US (UR), an approach response towards the context paired with the reward (place-preference) or away from the context paired with the punisher (place-aversion). Therefore, in place-preference, the CR is an incentive response to a distal CS much like the preparatory CR of Konorski (1967).

Place conditioning can be understood as a Pavlovian incentive response. The information obtained from place-conditioning studies is therefore complementary to those obtained from studies on PIT except that the conditioned approach or avoidance response to a Pavlovian stimulus (context) rather than the facilitation of instrumental responding by the noncontingent presentation of a Pavlovian stimulus is considered. In place conditioning, a performance effect of DA receptor blockers on the expression of the CR can be excluded by administering the drug only during acquisition. This arrangement does not exclude the possibility that the effects of the drug are due to failure to retrieve, in the absence of the drug state, the learned association formed under the drug state (state-dependency). This, however, can be controlled by the administration of the drug both in the acquisition and in the expression phase. Thus, in place-conditioning, a selective effect on acquisition can be accounted for by an action on the rewarding properties of the US or on the associative mechanism by which the context acquires conditioned incentive properties or on both.

Place conditioning has been widely utilized to investigate the role of DA in the action of drug and nondrug stimuli. DA receptor antagonists effectively impair place conditioning elicited by appetitive stimuli when given during conditioning. Thus, Spyraki et al. (1982) reported that haloperidol (0.1–0.2 mg/kg), given during conditioning to hungry rats, blocked the establishment of preference for the food paired compartment. The failure of Tombaugh et al. (1983) to impair by pimozide (1.0 mg/kg) the acquisition of incentive properties by a light or by a distinct compartment paired with food might be due to the fact that in that study rats were food deprived on test while they were fed ad libitum in the study of Spyraki et al. (1982). It is possible that in the study of Tombaugh et al. (1982), a deprivation state had enhanced the incentive properties of the food-paired environment to a degree sufficient to overcome any impairment of Pavlovian incentive learning during acquisition.

Impairment of the acquisition of place preference by DA receptor blockade could be due to reward devaluation or impairment of Pavlovian association. However the study of Agmo et al. (1995) shows that cis-flupenthixol blocks the acquisition of preference to a compartment paired to the drinking of 18% sucrose solution without reducing sucrose consumption. These results indicate that the impairment of place conditioning by DA receptor is related to the impairment of Pavlovian incentive learning rather than sucrose reward. Further studies show that raclopride, while not impairing lordosis behavior in female hamsters during sexual activity, prevents the establishment of preference for the place where sexual activity took place (Meisel et al., 1996). If lordosis behavior is taken as a measure of the hedonic impact of sexual activity, it appears that raclopride impairs Pavlovian incentive learning without reducing the rewarding impact of sexual stimulation. Similar conclusions were reached in studies of place preference conditioned by water drinking (Agmo et al., 1993). In this case both SCH 23390 (a D1 receptor blocker) and raclopride (a D2 receptor blocker) were able to impair place preference acquisition at doses that did not impair water drinking. Finally, SCH 23390 impaired at very low doses (0.01–0.03 mg/kg) the acquisition of place-preference conditioned by novel objects while it did not impair the interaction with novel objects (Besheer et al., 1999).

Under certain conditions, D2-specific neuroleptics, while ineffective per se, are able to facilitate place preference induced by food. These neuroleptics are sulpiride, pimozide and amisulpride while chlorpromazine, haloperidol and metoclopramide were ineffective (Guyon et al., 1993). These results can be explained by assuming that DA can inhibit its own activity, via D2-like DA receptors. Consistent with this, SCH23390 prevented this effect. In this study, amisulpride, given on test attenuated the effect of the same drug given during conditioning. Guyon et al. (1993) interpreted this observation as indicating that the impairment of associative learning was in part related to state-dependency. However, a more likely explanation is that amisulpride, given on test, impairs the expression of preference by impairment of performance.

A further example of the property of neuroleptics to impair the acquisition of incentive properties by stimuli paired with rewards is the observation that haloperidol (0.3 mg/kg) given during noncontingent electrical stimulation of the lateral hypothalamus prevented the establishment of conditioned preference for the compartment paired to the hypothalamic stimulation (Ettenberg and Duvauchelle, 1998). It is notable that in this study, hypothalamic stimulation was not contingent upon a subject response but was instead administered by the experimenter.

Summing up, impairment of DA transmission by DA receptor antagonists and in particular by D1 receptor antagonists impairs the acquisition of place preference conditioned by nondrug rewards (food, water, sucrose and sex). This effect is unrelated to an impairment of the hedonic impact of the rewards, consistent with other evidence, obtained from taste reactivity and consumption studies, that DA is not involved in stimulus-bound hedonia. Therefore, conditioned place-preference studies utilizing conventional rewards support a role of DA in Pavlovian incentive learning.

5.2.2.2. Dopamine and drug-conditioned place preference

The relative paucity of studies utilizing the place-conditioning paradigm for investigating the role of DA in the incentive properties of natural stimuli contrasts with the abundance of studies that have utilized this paradigm for investigating the incentive properties of drugs. The role of DA in drug-induced place preference has been investigated by drugs

interfering with DA transmission (e.g. neuroleptics) or with 6-OHDA lesions. As drugs or lesions affect DA transmission during the acquisition of place preference, its impairment can be the result not only of an impairment of incentive learning but also to a change in the rewarding properties of the drug.

Acquisition of amphetamine-induced place preference is reliably impaired by D1 (Leone and Di Chiara, 1987; Hoffman and Beninger, 1989) and D2 antagonists (Spyraki et al., 1982b; Mackey and Van der Kooy, 1985; Mithani et al., 1986) and by 6-OHDA lesions of the NAc (Spyraki et al., 1982b). Place-preference induced by cocaine administered i.p. is not affected by 6-OHDA lesions of the NAc (Spyraki et al., 1982b; Mackey and Van der Kooy, 1985) in spite of the ability of these treatments to impair cocaine-induced hypermotility (Spyraki et al., 1982b). Similar findings have been obtained in mutant mice that lack the D1 receptor gene or that do not express the D2-Long isoform. Thus D1-KO(−/−) (Miner et al., 1995) and D2L-KO (−/−) mice (Smith et al., 2002) show normal acquisition of CPP to i.p. cocaine in spite of the lack of cocaine-induced hypermotility. Since procaine also elicits a place preference resistant to neuroleptics (Spyraki et al., 1982b) it has been argued that place preference by i.p. cocaine is the expression of a non-DA effect dependent on some peripheral (intraperitoneal?) local anesthetic effect. For a discussion on this issue, see Di Chiara (1995).

Ethanol-induced place preference in DBA2 mice has been reported to be resistant to haloperidol, a preferential D2 antagonist (Risinger et al., 1992). Nicotine-induced place preference is prevented by a DI receptor antagonist (SCH 23390) (Acquas et al., 1989).

Opiate-induced place preference has been reported to be impaired by D2-preferring neuroleptics, D1 antagonists and 6-OHDA lesions of the NAc (Bozarth and Wise, 1981; Spyraki et al., 1983; Leone and Di Chiara, 1987; Shippenberg and Herz, 1987, 1988; Shippenberg et al., 1991, 1993). Other studies however, failed to observe an impairment of opiate place preference using a preferential D2 antagonist, such as alpha-flupenthixol (Mackey and Van der Kooy, 1985) and a specific D2 antagonist, such as sulpiride (Shippenberg and Herz, 1988; Shippenberg et al., 1993).

We have shown that morphine-induced place preference is reduced only by doses of D1 antagonists about 10 times higher than those that abolish amphetamine place preference (Acquas and Di Chiara, 1994). On this basis, we have suggested that the mechanisms by which D1 antagonists prevent amphetamine and morphine place preference are different, being related to reinforcer devaluation in the case of amphetamine and to the impairment of incentive learning in the case of morphine (Acquas and Di Chiara, 1994). It is interesting in this respect that D2L-KO mice, while acquiring CPP to i.p. cocaine, do not develop CPP to morphine (Smith et al., 2002). Similar effects have been obtained in homozygous mice bearing null mutations of the D2 gene (Maldonado et al., 1997) but not in mice with deletions of a sequence of the D2-gene (Dockstader et al., 2001).

The property of neuroleptics and DA D1 antagonists to impair the acquisition of drug-conditioned place preference has been taken by Beninger and associates (Beninger, 1991; Beninger and Miller, 1998; Sutton and Beninger, 1999) as evidence for a role of DA in incentive learning. However, most if not all drugs inducing place preference also increase extracellular DA in the NAc shell and this effect can, depending on the drug, contribute more or less substantially to its rewarding properties. Therefore, one cannot exclude that in the case of drug-conditioned place preference DA antagonists act by directly blunting reward rather than by impairing context-reward association. This possibility applies in particular to psychostimulants, that depend on the ability to increase DA in the NAc for most of their unconditioned effects, including the rewarding ones.

An exception, however, might be provided by aversive drugs, such as naloxone, lithium and picrotoxin for which an increase of DA in the NAc has not been observed (Bassareo et al., 1996). These drugs elicit place aversion that is blocked by the administration of the D1 receptor blockers SCH 23390 and SCH 39166 given during pairing with a specific compartment (Acquas et al., 1989). Similar considerations can be applied to the finding that haloperidol impaired the place aversion induced by a benzodiazepine inverse agonist (FG7142) known to induce anxiety but not convulsions in naive rats (Di Scala and Sandner, 1989). Moreover, Shippenberg and Herz (1987) reported that SCH 23390 blocks the establishment of place aversion to a k-opioid agonist, which is known to actually reduce DA release in the NAc. In relation to these studies, it is notable that SCH 23390, given in low doses, (12.5–25 mg/kg s.c.) induced place aversion for the compartment to which it had been paired (Acquas and Di Chiara, 1994). This observation might seem incompatible with the idea that blockade of D1 receptors impairs Pavlovian incentive learning. However, a higher dose of SCH 39166 (50 mg/kg s.c.) paired with both compartments prevented the establishment of place aversion induced by a dose of 12.5 mg/kg of the same drug (Acquas and Di Chiara, 1994). Thus, the doses of SCH 39166 needed to induce an aversive state are lower than those needed to impair Pavlovian incentive learning. This conclusion is consistent with the observation that low doses of SCH 39166 (12.5–25 mg/kg s.c.) are sufficient to impair conditioning to amphetamine while higher doses (50–100 mg/kg) are needed to impair place preference to morphine and place aversion to lithium (Acquas and Di Chiara, 1994). Thus, low doses of SCH 39166 block DA-dependent reward (amphetamine) than Pavlovian incentive learning (morphine and lithium).

Summing up, blockade of DA transmission impairs acquisition of place preference conditioned by appetitive as well as aversive drugs. In the case of psychostimulants this effect might be the result of a combination of an action on DA-dependent reward and on Pavlovian incentive learning. In the case of aversive drugs, which do not increase, or even decrease, DA transmission, an action on Pavlovian incentive learning is more likely.

5.2.2.3. Relative roles of NAc shell and core DA in place conditioning

Although the DA transmission of the NAc is commonly regarded as a critical substrate of the place preference conditioned by drugs of abuse, an analysis of the literature shows that this evidence derives essentially from the ability of 6-OHDA lesions to prevent acquisition of CPP following systemically administered drugs. Studies on the effect of the intracerebral infusion of DA receptor blockers are rare and limited to effects on expression rather than acquisition. Thus, Hiroi and White (1991) studied the effect of DA receptor antagonists on acquisition of amphetamine CPP only after systemic administration while testing the effect of their intracerebral infusion on expression. The reason for this is most likely that in expression studies a single intracerebral application of the antagonist is sufficient while acquisition studies require repeated intracerebral application during training with resulting diffusion of the antagonist to distant sites and loss of topographic selectivity. These difficulties add to the difficulties inherent in the drug-interaction nature of such studies. However, as already pointed out in this review, expression studies are critically affected by performance effects which raise doubts on their specificity. This flaw however does not seem to affect the studies by Baker et al. (1996, 1998) who showed that intraaccumbens sulpiride and SCH 23390 differentially affect acquisition of cocaine-conditioned place preference and cocaine-induced locomotion.

Thus, intraNAc infusion of sulpiride blocked locomotion but did not prevent CPP (Baker et al., 1996) while SCH 23390 prevented CPP but did not affect locomotion induced by cocaine (Baker et al., 1998). These observations are consistent with local infusion studies with amphetamine showing that intraNAc but not intraCPU infusion of amphetamine induce place preference (Carr and White, 1983, 1986; Hemby et al., 1992; Josselyn and Beninger, 1993; Schildein et al., 1998).

Although these studies did not attempt to differentiate between shell and core, most placements appear to be in the medial NAc; however, Schildein et al. (1998) explicitly targeted the NAc shell and observed an inverse relationship between rearing activity and acquisition of CPP with amphetamine. However, intraNAc cocaine fails to induce CPP up to 200–280 nmol (Hemby et al., 1992) but at 200 nmol induces CPP from the medial olfactory tubercle (Ikemoto, 2003). Local anesthetic effects are likely to affect the ability of intracerebral cocaine to induce CPP (Ikemoto, 2003).

As regards the relative role of the NAc shell and core in drug-conditioned place preference, the only study that has investigated this issue is a recent one by Sellings and Clarke (2003) who utilized 6-OHDA lesions and correlated the distribution of the loss of DA terminals as estimated by autoradiography of the DAT-ligand RTI-55 to changes in amphetamine-induced locomotion and acquisition of amphetamine and morphine-conditioned place preference. They found that reduction of amphetamine place preference correlated with loss of DA terminals in the NAc shell while reduction of locomotion correlated with loss of DA terminals in the NAc core. Lesions did not affect morphine-conditioned place preference, suggesting that the impairment of amphetamine CPP was due to an action on drug reward rather than on incentive learning.

Recently, we have completed a series of studies (Fenu et al., in preparation) comparing the effect of intra-NAc shell and core infusion of SCH 39166 and sulpiride on the acquisition and expression of place preference conditioned by morphine and by nicotine. In order to circumvent the difficulties inherent in the repeated intracerebral infusion of DA-receptor antagonists a single-trial paradigm of CPP was developed. The CPP consisted of testing for spontaneous preference in a two-compartment apparatus on the first day, association of the drug with the preferred side and of saline with the nonpreferred one on the second and third days, and testing for conditioned preference under extinction on the fourth day. The rats, implanted with guide cannulas aimed at the areas of interest, were infused with the antagonists or with saline immediately before the drug conditioning trial or before testing for conditioned preference. Doses of drugs effective in inducing single-trial CPP were 1 mg/kg sc of morphine. IntraNAc shell infusion of SCH 39166 dose-dependently impaired the acquisition of CPP to morphine (threshold dose, 12.5 ng/side) (Fig. 2). IntraNAc shell sulpiride also impaired the acquisition of place preference conditioned by morphine (threshold dose 25 ng/side) (Fig. 3). IntraNAc core infusion of SCH 39166 up to doses of 50 ng/side, failed to affect acquisition of CPP (Fig. 2). Sulpiride in the NAc core impaired CPP to morphine at doses of 50 ng/side (Fig. 3). IntraNAc shell infusion of SCH 890166 and sulpiride, up to doses of 25 ng/side, failed to impair expression of CPP. Doses of sulpiride of 50 m/side in the NAc core impaired the expression of morphine CPP but also induced impaired locomotion. These results, while confirming previous observations on the role of DA in the acquisition of morphine and nicotine CPP, point to the NAc shell as the critical site for this role. As to expression of CPP, these results are consistent with results of other studies (e.g. McFarland and Ettenberg, 1995) indicating that DA does not play a critical role in the expression of simple incentive responses.

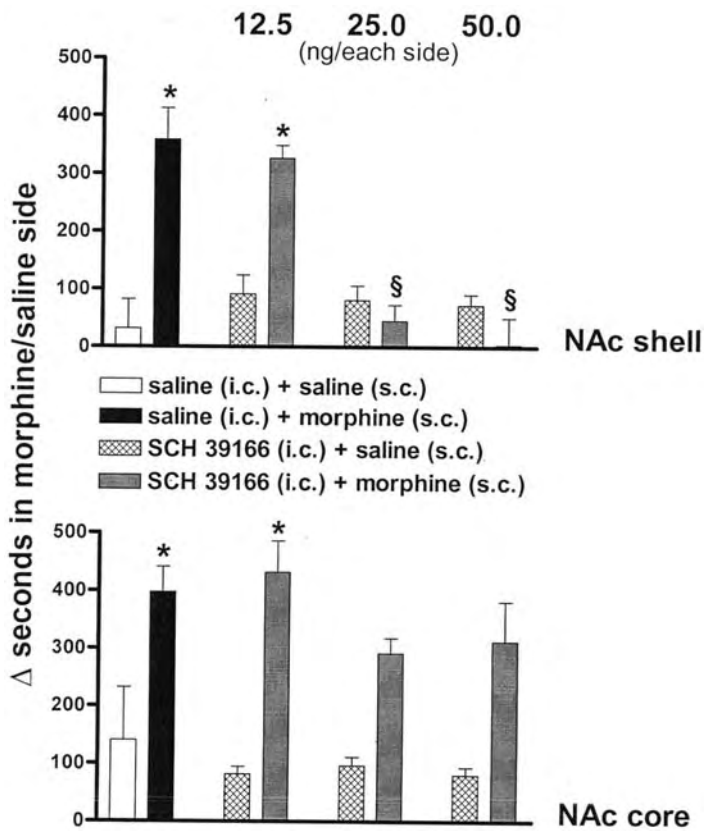

Fig. 2. Effect of NAc shell and core DA D1 receptor blockade on acquisition of morphine-conditioned place preference. SCH 39166 (12.5, 25.0 and 50.0 ng / side), or saline was infused into the NAc shell (upper panel) and core (bottom panel) 10 min before morphine (1 mg/kg s.c.). Each bar represents mean ± SEM of the difference (in seconds) between the time spent in the drug-paired compartment and the time spent in the same compartment in the preconditioning session. $*P < 0.05$ versus saline s.c. of the corresponding control group; § $p < 00.5$ versus saline i.c. + morphine s.c.

5.2.2.4. Role of the n.accumbens shell DA in conditioned taste aversion learning

Conditioned taste aversion (CTA) is a special form of Pavlovian learning having the advantage that efficient association takes place even after a single trial and that a long-interval (up to 6 h) can be allowed between presentation of the gustatory CS and the US. This interval is consistent with the function of this associative mechanism, which relates to avoidance of foods whose harmful effects are experienced only following adequate digestion. During this interval a short-term memory trace of the CS has to be formed and consolidated in order to allow subsequent association of the CS with the US (Bures et al., 1988). Therefore, by applying drugs during this interval, learning can be manipulated without interfering with the impact of the CS or with the reinforcing properties of the US. Findings generally consistent with a lack of impairment of DA in CTA learning have been reported by Berridge and Robinson (1998) in a CTA paradigm utilizing taste reactivity as a means to estimate the affective properties of the taste stimulus. 6-OHDA lesions that reduced by more than 98% DA in the neostriatum and by 85–99% DA in the

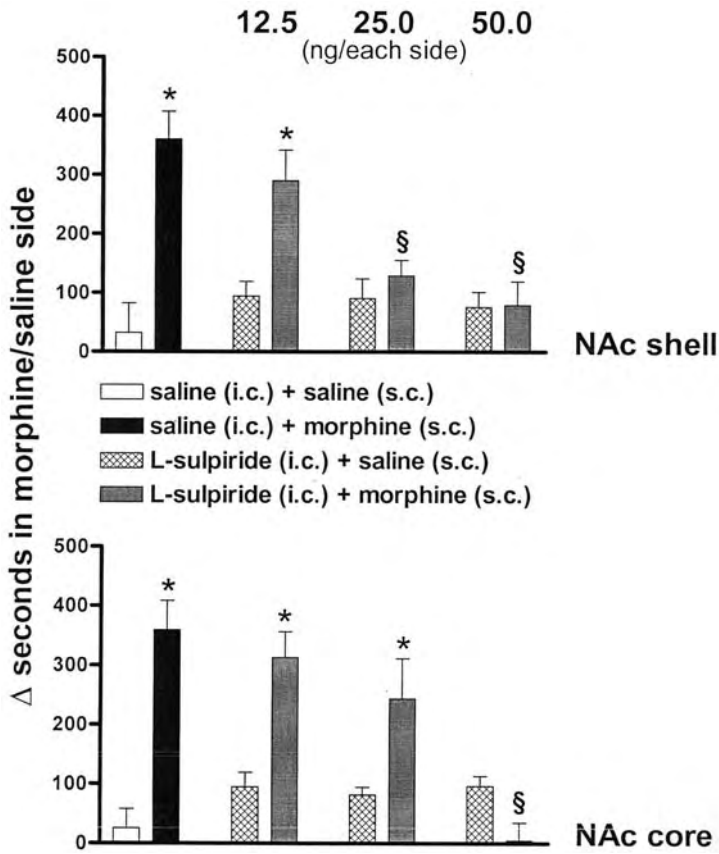

Fig. 3. Effect of NAc shell and core DA D2 receptor blockade on acquisition of morphine-conditioned place preference. L-sulpiride (12.5, 25.0 and 50.0 ng/ side), or saline were infused into the NAc shell (upper panel) and core (bottom panel) 10 min before morphine (1 mg/kg s.c.). Each bar represents mean ± SEM of the difference (in seconds) between the time spent in the drug-paired compartment on the testing day and the time spent in this compartment in the preconditioning session. $*P < 0.05$ versus saline s.c. of the corresponding control group; § $p < 00.5$ versus saline i.c. plus morphine s.c.

NAc did not impair the acquisition of aversive taste reactions to intraoral sucrose previously paired with intraperitoneal lithium-induced malaise. In contrast with the above lesion studies, studies utilizing acute blockade of DA transmission by DA receptor antagonists provide evidence for a role of DA D1 receptors in CTA learning (Fenu et al., 2001). Systemic administration of the D1 receptor antagonist SCH 23390 5 min after exposure to the appetitive taste stimulus (sucrose or saccharin) during single-trial pairing with lithium as the US results in reduction of CTA (as indicated by an increase in the amount of sucrose or saccharin solution ingested from bottles) on a subsequent test performed in the absence of the D1 antagonist (Fenu et al., 2001). The effect of systemic SCH 23390 on CTA learning was confirmed by utilizing a taste reactivity paradigm evaluating the changes in hedonic reativity to the intraoral infusion of a chocolate-sucrose solution, induced by lithium pairing. Thus, SCH 23390, given on conditioning trials 5 min after the intraoral infusion of chocolate-sucrose solution, reduced the conditioned aversive reactions elicited on a subsequent test by the same solution.

The effect of systemic SCH 23390 could be reproduced by local infusion of the more selective D1 antagonist SCH 39166 in the NAc shell and to a lesser extent in the lateral hypothalamus, a DA-rich area that receives direct projections from the NAc shell. These observations in turn confirm and extend previous observations by Caulliez et al. (1996) that infusion of SCH 23390 in the lateral hypothalamus impairs CTA learning. No effects were obtained from the NAc core nor from the bed nucleus of stria terminalis. The action of the D1 antagonist was time-dependent, since it did not take place when the D1 antagonist was given 45 min instead of 5 min after the CS or at various time intervals before it. These characteristics are consistent with the idea that the D1 antagonist acts at a time critical for the formation and consolidation of the short-term memory trace of the CS. These observations therefore suggest a role of DA in the formation and consolidation of a short-term memory trace of novel gustatory stimuli (Fenu et al., 2001). This mechanism might be coupled to the release of DA in the NAc shell by novel appetitive stimuli. Thus, appetitive taste stimuli release DA in the shell and this response undergoes single-trial habituation (Bassareo and Di Chiara, 1997, 1999a,b; Bassareo et al., 2002). Systemic administration of amphetamine facilitates sucrose-conditioned CTA and this effect is blocked by intrashell infusion of SCH39166 (Fenu and Di Chiara, 2003). Various studies have shown that stimulation of DA receptors in the matrix of the dorsal striatum facilitates memory consolidation of stimulus/response associations (habits) (White, 1997). On the other hand, stimulation of D2-like receptors in the amygdala has been reported to facilitate consolidation into long-term memory of Pavlovian stimulus-reward associations (Hitchcott et al., 1997a,b; Hitchcott and Phillips, 1998). More recently, a role of NAc shell DA similar to that of the central amygdala in consolidation of Pavlovian associations in an autoshaping paradigm has been reported (Phillips et al., 2003). These observations, however, are different from those obtained in CTA studies (Fenu et al., 2001) since they refer to consolidation into long-term memory of the CS-US association rather than to formation and consolidation of a short-term memory trace of the CS.

5.3. N. ACCUMBENS SHELL DOPAMINE AND THE UTILIZATION OF SPATIAL MEMORY FOR GOAL-ORIENTED BEHAVIOR

Recent studies by Floresco et al. (1996, 1987) have demonstrated a role of endogenous DA acting on D1 receptors in the use of hippocampal spatial memory for efficient foraging in a radial eight arm maze. Rats are trained to efficently forage by entering only once in the baited arms of the maze (win/shift). Two tasks are utilized, a single step, nondelayed task (random foraging) and a two step, 30 min delay task. In order to perform in the nondelayed task rats have to progressively store into short-term memory spatial information about the location of the baited arms while exploring the maze and utilize it to avoid those already visited. In this way behavior is guided by a form of spatial working memory of recently visited arms. In the delayed two-step task, rats utilize spatial information acquired during the first step to guide their strategies for correct performance in the second step. Bilateral infusion of a D1 antagonist, but not of a D2 antagonist, in the medial NAc (correspondent to the NAc shell) resulted in inefficient foraging in the nondelayed task. These effects were mimicked by infusion of a D1 antagonist in the medial NAc of one side associated to inactivation of the ventral subiculum of the opposite side by local perfusion with lidocaine. In contrast, the same manipulations did not impair prospective foraging in the delayed task. Performance in the delayed task but not

in the nondelayed one was impaired by bilateral intra-PFCX (prelimbic cortex) infusion of a D1 antagonist or by unilateral infusion associated to inactivation of the contralateral ventral subiculum (Seamans et al., 1998).

These studies show that memory-guided foraging in the radial maze is related to activity in a distributed network including the hippocampal formation (ventral subiculum), which sends direct projections to the NAc shell and to the prelimbic PFCX, which in turn directly projects to the NAc. DA, acting on D1 receptors, modulates the trasfer of information at two critical sites, the PFCX and the NAc. In the PFCX, DA enables the prospective use of delayed spatial information. In the NAc, DA enables the retrospective use of spatial information acquired during exploratory activity.

These observations, while fully consistent with the general role of NAc shell DA in incentive arousal, specify an important function of DA in this area in the utilization of short-term memory about the unpredicted location of reward for efficient goal-directed behavior.

5.4. N.ACCUMBENS CORE DOPAMINE AND ACQUISITION OF INSTRUMENTAL RESPONDING

Various studies show that lesion or reversible impairment of excitatory transmision of the NAc core impair acquisition of instrumental responding for food and for drug self-administration. The mechanism of this effect, however, is debated.

Kelley et al. (1997) initially showed that intraNAc core infusion of AP5, an NMDA receptor antagonist, impaired the acquisition of bar-pressing for food but did not affect responding once the instrumental response had been acquired. Later on this observation was extended to other brain areas like the basolateral amygdala and the PFCX as part of a distributed network including also the postero-medial and the dorsolateral striatum (Baldwin et al., 2000). Activity within this network was suggested to provide the neural substrate for learning of instrumental action. This group also showed (Smith-Roe and Kelly, 2000) that intraNAc core coinfusion of a D1-receptor blocker and an NMDA antagonist at doses that are ineffective by themselves, impair the acquisition of instrumental responding (Baldwin et al., 2002). Similar observations have been made in the PFCX and in the amygdala (Kelley, 2004).

These observations have been interpreted to suggest that the acquisition of instrumental responding depends on a permissive influence of DA on postsynaptic NMDA receptor-mediated actions of glutamate released from excitatory input to the NAc core originating from the PFCX and from the basolateral amygdala (Kelley, 2004). In this way, DA would gate executive input to the NAc core arising from the PFCX and information over the hedonic value of the outcome arising from the basolateral amygdala.

Excitotoxic NAc core lesions slow down the acquisition of first order schedules of heroin self-administration but do not affect already established responding (Alderson et al., 2001; Hutcheson et al., 2001). An impairment of instrumental learning by these lesions has been excluded on the basis of the observation that responding was still sensitive to changes in the outcome value. On the basis of the above studies, Cardinal et al. (2002) maintain that the NAc core is not critical for instrumental learning and interpret the effects of NAc core lesions in acquisition as the result of an impairment of the motivational arousal elicited by drug-conditioned stimuli. Consistent with this explanation is the observation that NAc core lesions prevent acquisition of second-order schedules

of drug self-administration, a task highly dependent on the incentive arousing influence of drug-conditioned stimuli (Hutcheson et al., 2001). As to the effect of reversible manipulations of NAc core functions on acquisition of responding for food, Cardinal et al. (2002) implicate a degradation of act–outcome contingency due to slowing of food approach during conditioning.

5.5. DISSOCIABLE FUNCTIONS OF DA IN THE N.ACCUMBENS CORE AND SHELL IN INSTRUMENTAL RESPONDING FOR FOOD

IntraNAc infusion of drugs acting on DA (amphetamine), opiate (DAMGO) and GABA receptors (muscimol) differentially affect free feeding, taste reactivity, and progressive ratio responding in an already acquired instrumental lever pressing task and acquisition of a new instrumental lever-pressing task for food (Hanlon et al., 2004). IntraNAc shell muscimol and DAMGO but not amphetamine increase free feeding in fed ad lib rats (Stratford and Kelley, 1997, 1999; Zhang and Kelley, 2000; Kelley, 2004). IntraNAc shell DAMGO but not muscimol and amphetamine increase hedonic reactions to food taste (Pecina and Berridge, 2000). Local DAMGO and amphetamine increase breaking point in progressive ratio responding (Hanlon et al., 2004). None of the agonists infused in the NAc shell facilitate acquisition of instrumental responding for food (Hanlon et al., 2004). Hunger typically increases behavioral measures in all the above tasks (Hanlon et al., 2004). It appears therefore that none of the manipulations are able to mimic the properties of hunger. However, each manipulation separately affects specific elements of the effect of hunger on the impact of food on instrumental responding. Thus, intraNAc shell DAMGO increases the hedonic value of food taste while amphetamine increases the incentive properties of stimuli conditioned to food (discriminative stimuli, food-conditioned reinforcers, Pavlovian food-conditioned stimuli etc) (Hanlon et al., 2004). The observation that both these manipulations increase already acquired progressive ratio performance indicates that this paradigm does not differentiate between changes in reward value and changes in motivational (incentive) value of stimuli. Nonetheless, under sated conditions, facilitation of the incentive impact of food-associated stimuli by intraNAc amphetamine, or increase in the hedonic value of the reward by local DAMGO, is insufficient to reproduce the ability of hunger to promote instrumental responding (Hanlon et al., 2004). It is unknown if combined intraNAc shell infusion of amphetamine and DAMGO reproduce the properties of hunger in sated rats.

5.6. DOPAMINE AND DRUG REWARD AND REINFORCEMENT

Drugs abused by humans are self-administered by experimental animals (Yanagita, 1973; Johanson, 1978; Pickens et al., 1978; Johanson and Schuster, 1981; Markou et al., 1993). Drug self-administration however, is only an expression of the reinforcing properties of drugs and not of its addictive properties; nonetheless, it is provided with good predictive and partial construct validity also for addiction. Thus, a drug that is self-administered by animals can be predicted to have addictive liability. Therefore, the property of serving as a reinforcer seems necessary for a drug to be addictive. Based on these grounds, results obtained with the self-administration paradigm are particularly

important for current thoughts on the role of DA in the reinforcing properties of drugs of abuse and in the mechanism of drug abuse.

5.6.1. Interpretation of changes in rates of drug self-administration

In spite of its apparent straightforwardness, drug self-administration is affected by serious interpretative difficulties that limit its usefulness in the study of the basic mechanisms of drug motivated behavior. These difficulties are mainly related to the biphasic nature of the dose-effect relationship. In the following example we will consider the case of cocaine i.v. self-administration.

In fixed-ratio schedules, including CRF (continuous reinforcement) schedules, the relationship between unit dose of cocaine and response rate is bell-shaped (Pickens and Thompson, 1968; Goldberg et al., 1971b; Wilson et al., 1971). At low unit doses, responding increases as unit dose is increased, being a direct function of the rewarding value of the drug. As peak rates of responding are reached, further increase in unit dose does not result in a comparable increase in response rate; actually, rate of responding decreases as the dose increases. This behavior can be explained by postulating the existence of an optimal steady-state concentration of psychostimulant that maximally stimulates responding (reinforcing window); once this level is reached, further increase in psychostimulant dose would be compensated by a reduction in rate of responding (Yokel and Pikens, 1974). According to this interpretation, reduction in response rate at high doses of cocaine is the effect of the increase in unit dose rather than the result of a rate decreasing action of the drug (as argued instead by Herling and Woods, 1980). This interpretation is confirmed by the fact that, even in the descending limb of the dose-response curve, drug intake increases when it is normalized for the amount of drug self-administered (responses/mg) (Pettit and Justice, 1991); moreover, introduction of an appropriate time-out period after each administration (Griffiths et al., 1979) or the use of interval schedules (Balster and Schuster, 1973b), which limits the possibility of a rate-dependent build-up of drug concentrations, result in a linear dose-response relationship over a range of doses that would result in a bell-shaped relationship on the conventional FR schedules. Finally, unit doses of cocaine that reduce responding on the conventional FR schedules do result in a linear dose-response curve when the strength of cocaine reinforcement is evaluated by obtaining for each dose the breaking point (Hodos, 1961) on a progressive ratio schedule (i.e. the maximal ratio at which the schedule is completed) (Griffiths et al., 1979).

The concept of a reinforcing window, i.e. of an optimal steady-state level of drug taken by the subject as reference for adjusting its rate of responding to the unit dose, involves the assumption that there is a saturation limit in the positive reinforcing properties of a drug. Indeed this property is not exclusive to drugs as other positive reinforcers such as sweet (Towell et al., 1987; Muscat and Willner, 1989; Phillips et al., 1991b,c,d) and ICSS (Hodos and Valenstein, 1962) show similar bell-shaped dose-response relationships.

5.6.2. Psychostimulant self-administration

The above framework forms the basis for interpreting the effect of experimental manipulation of DA transmission on responding for cocaine self-administration. Neuroleptics reduce responding on the ascending limb of the bell-shaped dose-response curve while they increase responding on the descending limb (Wilson and Schuster, 1972;

de Wit and Wise, 1977; Ettenberg et al., 1982; Roberts and Vickers, 1984; Koob et al., 1987; Bergman et al., 1989; Britton et al., 1991). This effect results in an overall shift to the right in the dose-response function, consistent with a competitive antagonism and with self-titration of cocaine around optimal concentrations in the blood (Bergman et al., 1990; Mello and Negus, 1996; Caine et al., 2002). Quite a different pattern of change has been observed in D2 KO (knockout) mice by Caine et al. (2002). Thus, in D2 KO mice cocaine is administered at similar rates as in the wild type in the ascending limb of the dose-effect curve and at higher rates in the descending limb. This asymmetric shift is the exception rather than the rule after neuroleptics (Woods et al., 1978). Apart from the arguments put forward to explain these observations (Woods et al., 1987; Caine et al., 2002), the striking differences between the effects of acute pharmacological inactivation of D2 receptors and their genetic deletion indicate that in the mutants an extensive degree of compensatory changes take place.

Consistent with a reduction of cocaine reinforcement as the mechanism of the neuroleptic-induced increase in responding for cocaine in FR schedules, is the observation that the same doses of haloperidol and of antagonists specific for D1 or D2 receptors that increase responding on a FR schedule also reduce the breaking point of cocaine self-administration on a progressive ratio schedule (Roberts et al., 1989; Hubner and Moreton, 1991). Further evidence for a role of DA in cocaine reinforcement has been provided in the monkey, in a multiple schedule of drug and food reinforcement, where low concentrations of the D1 antagonist SCH 23390 continuously infused for 24 h, selectively reduced responding for cocaine on a lean schedule of responding (FR 30) (Kleven and Woolverton, 1990) in which, in a previous study, no cocaine-specific effect could be demonstrated after the bolus administration of pimozide or SCH 23390 (Woolverton, 1986). Notably, in the monkey, at variance with the rat (Koob et al., 1987), SCH 23390 fails to increase responding on FR schedules with (Woolverton and Virus, 1989) and without (Woolverton, 1987) a time-out period; moreover, acute SCH 23390 (Woolverton and Virus, 1989) and neuroleptics (Woolverton and Balster, 1981) fail to specifically impair cocaine reinforcement as they also reduce responding for food.

Evidence for a role of NAc DA in cocaine reinforcement has been provided by studies of the effect of 6-OHDA lesions on cocaine self-administration. Thus, 6-OHDA lesions of the DA innervation of the nucleus accumbens result in extinction-like effects on responding for cocaine in FR schedules (Roberts et al., 1977; Roberts et al., 1980, Pettit et al., 1984) and in a decrease in break point in progressive ratio schedules (Koob et al., 1987). In contrast, 6-OHDA lesions of the prefrontal cortex, an area where cocaine is self-infused (Goeders and Smith, 1983; Goeders et al., 1986), fail to reduce (Martin-Iverson et al., 1986) or actually increase (Schenk et al., 1991) cocaine self-administration.

Studies with amphetamine also show extinction-like effects of neuroleptics, with an increased rate of responding followed by a reduction to saline values (Balster and Schuster, 1973a; Yokel and Wise, 1975, 1976; Risner and Jones, 1976). Extinction of responding for amphetamine is also specifically induced by 6-OHDA lesions of the DA innervation of the NAc (Lyness et al., 1979).

5.6.3. Opiate self-administration

In order to distinguish specific effects on reinforcement from nonspecific effects on performance various studies on the effect of DA receptor blockers and 6-OHDA lesions on opiate self-administration have looked for an increase in responding in fixed-ratio

schedules for doses of opiate in the descending limb of the bell-shaped dose response function. Consistent with a reduction of opiate reward, administration of opiate antagonists increases responding and, at high doses, elicits extinction-like effects with return to saline values after a burst of increased responding (Goldberg et al., 1971a; Weeks and Collins, 1979; Ettenberg et al., 1982).

In one of the early studies on this issue, the effect of α-flupentixol, a D2-DI DA receptor antagonist, was studied in rats independently self-administering cocaine and heroin on a FR schedule (Ettenberg et al., 1982). The neuroleptic, in low doses, increased cocaine self-administration while at higher doses reduced responding. No such pattern was observed for the effect of neuroleptic on heroin self-administration; thus, doses of α-flupentixol that increased cocaine responding failed to affect heroin responding and the neuroleptic decreased heroin responding only at doses that decreased altogether cocaine responding (Ettenberg et al., 1982). In a subsequent study, it was shown that 6-OHDA lesions of mesolimbic DA neurons that progressively reduce cocaine responding across sessions, result in a progressive recovery of heroin responding to normal rates after few sessions (Pettit et al., 1984).

Studies with complex schedules of multiple reinforcement for drug, food and water have similarly shown that 6-OHDA lesions that shift to the right the dose-response function for cocaine, do not affect that for opiate or food responding (Dworkin et al., 1988) thus contradicting an earlier study reporting an impairment of opiate self-administration by 6-OHDA lesions (Smith et al., 1985).

In contrast with the reports that neuroleptics and 6-OHDA lesions impair acquisition of place preference conditioned by opiates, DA receptor blockers failed to impair the acquisition of opiate self-administration except at doses that nonspecifically impaired responding (Van Ree and Ramsey, 1987; Gerrits et al., 1994).

In D2 KO mice conflicting results have been obtained on a place preference paradigm. In morphine-naïve mice Maldonado et al. (1997) reported an impairment of morphine-conditioned place preference while Dockstader et al. (2001) did not. Curiously morphine-conditioned place preference was impaired in morphine-dependent D2 KO mice.

Genetic deletion of D2 receptors disrupted instrumental responding for i.v. morphine (Elmer et al., 2002). However the specificity of this effect is questionable as the acquisition of instrumental responding for water was also reduced.

Therefore, self-administration studies do not provide any evidence for a specific role of DA in opiate reinforcement and on this basis it has been concluded that DA does not play a role in the reinforcing properties of opiates (Ettenberg et al., 1982; Pettit et al., 1984; Van Ree and Ramsey, 1987; Gerrits et al., 1994).

5.6.4. Nicotine self-administration

The role of DA in the reinforcing properties of nicotine has been investigated by studying the effect of selective D1 and D2 antagonists and of 6-OHDA lesions on a FR schedule of i.v. self-administration of nicotine in comparison with responding for food (Corrigall and Coen, 1991; Corrigall et al., 1992). Both DI and D2 antagonists and 6-OHDA lesions reduced response rates for i.v. nicotine self-administration and for food presentation. Neuroleptics were effective at doses lower than those that impair locomotor stimulation by nicotine (Corrigall and Coen, 1991). While high doses of neuroleptics reduced responding from the beginning of the session, low doses reduced responding at the end of the session, in an extinction-like fashion. Failure to obtain an increased responding for nicotine after

DA receptor blockade is explained as due to the direct (rather than inverse) relationship between unitary dose of nicotine and response-rate in the dose-range selected for these experiments (ascending limb of the dose-effect function) (Corrigall and Coen, 1991). In fact, direct blockade of nicotine action at its receptor by mecamylamine or by chlorindosamine reduced the rate of responding for nicotine (Corrigall and Coen, 1991).

Given the ability of neuroleptics and 6-OHDA lesions to impair responding for nicotine and for food, it is unclear to what extent their effect on nicotine self-administration is specifically related to an impairment of nicotine reinforcement.

5.6.5. Ethanol self-administration

The study of the role of DA in ethanol self-administration is affected by problems not dissimilar from those encountered with other drugs of abuse except that, in contrast to the fair consistency in one direction or in the other among studies on psychostimulants and opiates, much disagreement is registered in the case of ethanol.

An additional problem with these studies might result from the fact that ethanol preference over water, although utilized for investigating the reinforcing properties of ethanol, may not be a correlate of such properties (George, 1990; Cunningham et al., 1992).

In an early study of continuous access to ethanol, pimozide failed to alter drinking of ethanol or water (Brown et al., 1982). Linesman (1990), on a limited access paradigm, observed a reduction by haloperidol of ethanol drinking at doses that also impaired water drinking. Pfeffer and Samson (1985, 1986), in rats trained to drink consistent ethanol amounts by a sucrose-fading procedure, showed that pimozide reduced operant responding for ethanol drinking as well as nonoperant drinking of ethanol only when ethanol availability was restricted to a 30-min session but not when unrestricted. In agreement with the above studies, Dyr et al. (1993) showed that SCH 23390 reduced ethanol drinking on a limited access paradigm in ethanol preferring rats and Rassnick et al. (1992) observed a reduction of operant responding for ethanol drinking after fluphenazine, a D2–D1 antagonist, in rats that acquired ethanol preference by a saccharin fading technique on a limited access paradigm. The above studies have been complemented by the observation that local intraaccumbens infusion of DA receptor antagonists reduce operant responding for ethanol in rats with acquired ethanol-preference (Rassnick et al., 1992; Samson et al., 1993). In rats selectively bred for ethanol-preference (P rats) and drinking ethanol from bottles, Levy et al. (1991) showed an increase in ethanol drinking after intraaccumbens sulpiride. The results of a study by Lyness and Smith (1992) have not been taken into consideration in view of the exceedingly low unit doses of ethanol (< 5 mg) that maintained intravenous self-administration in rats.

From these studies it appears that in rats with a high ethanol intake obtained by selective breeding or by the sweet-fading techniques and on a limited access paradigm of operant ethanol self-administration, neuroleptics consistently reduce responding for ethanol. This effect seems specific for ethanol, since responding for water was unimpaired.

The interpretation of the mechanism of the influence of neuroleptics on ethanol reinforcement and the appraisal of the precise role of DA is complicated by the fact that DA receptor agonists either direct (e.g. bromocriptine) or indirect (e.g. amphetamine), similarly to neuroleptics, reduce ethanol drinking (Samson et al., 1993).

The results of studies on the effect of 6-OHDA lesions on ethanol reinforcement are largely negative (Kiiaanmaa et al., 1979; Rassnick et al., 1993) apart from one study showing an increase in ethanol drinking after intraaccumbens 6-OHDA (Quartford et al., 1991) and an earlier one showing a decrease after i.c.v. infusion of 6-OHDA (Myers and Melchior, 1975). However, the specificity of these lesions was not investigated.

In conclusion, DA might play a role in ethanol reinforcement although the specificity of this role is unclear.

5.6.6. Role of dopamine in psychostimulant versus conventional and non-psychostimulant reinforcement

From the above analysis it appears that the effect of impairment of DA transmission by neuroleptics and 6-OHDA lesions of DA terminals in rats and genetic deletion of DA receptors on drug reinforcement widely differs depending on the specific drug class. From this point of view, drug rewards can be distinguished into two broad categories: psychostimulants, such as cocaine and amphetamine, and nonpsychostimulants, such as heroin, nicotine and ethanol. In the rat and in the mouse, striking differences are observed between the effects of impairment of DA transmission on psychostimulant reinforcement, and nonpsychostimulant reinforcement and food reinforcement. Thus, doses of the DA receptor antagonists that markedly impair cocaine self-administration leave intact food (Roberts et al., 1977, 1980; Caine and Koob, 1994a) and heroin self-administration (Pettit et al., 1984).

5.6.6.1. Role of n.accumbens dopamine in psychostimulant reinforcement

A role of NAc DA in cocaine reinforcement is indicated by the observation that 6-OHDA lesions of the NAc impair i.v. amphetamine (Lyness et al., 1979) and cocaine, but not heroin self-administration (Pettit et al., 1984). Genetic deletion of the DA transporter (DAT) fails to impair cocaine self-administration (Rocha et al., 1998) and cocaine-conditioned (Sora et al., 1998) as well as amphetamine-conditioned (Budygin et al., 2004) place preference. However, cocaine and amphetamine, while failing to increase extracellular DA in the dorsal striatum, do increase extracellular DA in the NAc of of DAT-KO mice (Carboni et al., 2001; Budygin et al., 2004) and this effect is mimicked by reboxetine, a specific blocker of the NE transporter (NET) (Carboni et al., 2001). These observations indicate that in the DAT-KO mice cocaine and amphetamine are reinforcing because they increase DA in the NAc. However, both cocaine (Budygin et al., 2002) and amphetamine (Budygin et al., 2004) fail to affect the clearance of DA, as estimated by fast cyclic voltammetry, when applied to NAc slices from DAT-KO mice. On the one hand, local intraaccumbens cocaine (Mateo et al., 2004) and amphetamine (Budygin et al., 2004) fail to increase DA in the NAc of DAT-KO mice. Moveover, systemic citalopram and fluoxetine increase DA in the NAc of DAT-KO mice (Mateo et al., 2004). On this basis it has been suggested that cocaine and amphetamine increase DA in the NAc of DAT-KO mice by increasing 5HT in the VTA. Consistent with this hypothesis, intraVTA fluoxetine increases DA in the NAc of DAT-KO mice (Mateo et al., 2004). Moreover, amphetamine, while inhibiting the firing activity of DA neurons in the VTA, stimulated it in DAT-KO mice (Budygin et al., 2004). Furthermore, blockade of 5HT1A receptors, prevented both the increase of DA in the NAc as well as the CPP induced by amphetamine in DAT-KO mice (Budygin et al., 2004). Thus, the increase

of DA in the NAc of DAT-KO mice induced by cocaine and amphetamine might be related to a 5HT-mediated stimulation of the firing of DA neurons in the VTA. However, a role of 5HT in the rewarding properties of cocaine in DAT-KO mice is in contrast with the observation of Rocha (2003) that cocaine is self-administered by DAT-KO mice also in the presence of fluoxetine. Although further studies are needed to explain the exact mechanism of cocaine reinforcement in the DAT-KO model, these studies confirm the role of NAc DA in cocaine and amphetamine reward and reinforcement.

5.6.6.2. The n. accumbens shell as the primary site of psychostimulant reinforcement

Intracerebral self-administration studies strongly support the notion that the NAc shell is the primary site of action of the reinforcing actions of direct DA receptor agonists and psychostimulants (see McBride et al., 1999 for review). Thus, D1 and D2 agonists are coinfused in the NAc shell (Ikemoto et al., 1997). Infusion of D1 or D2 agonists alone in the NAc shell or coinfusion of D1 and D2 agonists in the NAc core did not support self-infusion (Ikemoto et al., 1997). Amphetamine (Hoebel et al., 1983), nomifensine (Carlezon et al., 1995) and phencyclidine (Carlezon and Wise, 1996) are self-infused by rats in an area of the NAc that corresponds to the shell. The same doses of amphetamine were ineffective when infused in the dorsal striatum (Hoebel et al., 1983). Phillips et al. (1994) reported self-infusion of amphetamine in the NAc core, near the border with the shell. Chevrette et al. (2002) recently reported a bell-shaped dose-effect curve for cocaine self-infusion in the NAc shell and in the central amygdala. Unfortunately, in this study a direct comparison of the responsiveness of NAc shell and core was not performed. In contrast with the failure of Goeders and Smith (1983) to obtain self-infusion of cocaine in the NAc, Carlezon et al. (1995) and McKinzie et al. (1999) obtained self-infusion of cocaine from the NAc shell. On the other hand, Caine et al. (1995) have reported that local intraNAc shell and intraamygdala infusion of SCH 23390 increases responding to a similar extent as systemic SCH 23390; infusions in the dorsal caudate-putamen were ineffective.

Recently, a direct comparison between shell and core in the ability to maintain local self-infusion of cocaine in a two-lever apparatus has been performed by Rodd-Henricks et al. (2002). Cocaine was self-infused in the shell in a dose-related fashion by pressing on the active lever. Neither self-infusion from the NAc core nor from the sites ventral to the shell could be evoked, correspondent to the medial olfactory tubercle (OT). In partial contrast with these observations, Ikemoto (2003) has recently reported that cocaine is self-infused at higher rates in the antero-medial OT than in the postero-medial OT and in the medial NAc shell, but is not self-infused in the NAc core or in the central caudate-putamen. Given the topographical relationships between the medial OT and the medial NAc shell and the expected diffusion of a lipophilic drug like cocaine after repeated intracerebral infusion, it is technically difficult to attribute specifically to the OT and not to the adjacent NAc shell the effects observed. Previous studies by the same author had shown that D1 and D2 agonists are coinfused in the NAc shell (Ikemoto et al., 1997). Infusion of D1 or D2 agonists alone in the NAc shell or coinfusion of D1 and D2 agonists in the NAc core did not support self-administration (Ikemoto et al., 1997).

An additional site of cocaine self-infusion is the prefrontal cortex (Goeders and Smith, 1993; Carlezon and Wise, 1996). However this effect may not be related to DA as amphetamine is not self-infused in this area.

5.6.6.3. Psychostimulant reward as dopamine dependent state-hedonia

Activational hypotheses of DA function, having been developed as alternative and often in opposition to the anhedonia hypothesis, have assumed that not only food but also drug-induced reward, including psychostmulant reward, is unrelated to stimulation of DA transmission. This position has been very explicitly expressed by Robinson and Berridge (1993) who have proposed that, even in the case of psychostimulants, DA mediates 'wanting' (also termed 'incentive salience') but not 'liking'. These hypotheses however do not account for the fact that stimulation of DA transmission itself has a positive unconditioned motivational valence. Why, for example, are DA agonists readily and consistently self-administered in the nucleus accumbens by naive animals? Why do animals consistently approach stimuli that have been paired with amphetamine and avoid stimuli that have been paired with a D1 receptor antagonist (Acquas and Di Chiara, 1994)? These observations, which cannot be explained by the activational hypothesis, can be interpreted as indicating that changes in DA transmission in specific brain areas affect the valence of the motivational state. Salient stimuli neutral to their motivational valence are assigned the motivational valence of the state under which they are experienced; with this premise, stimuli experienced under an increased DA transmission would be assigned a positive motivational valence and therefore act as positive reinforcers while stimuli experienced under a decreased DA transmission would be assigned a negative motivational valence and therefore act as negative reinforcers.

The main conceptual difficulty for accepting the idea that DA can possess an intrinsic hedonic value derives from the assumption, most likely correct, that food reward is not dependent on DA (Berridge, 1996; Salamone et al., 1997).

We believe that a correct interpretation of the role of DA in reward should start from acknowledging the differences between food and psychostimulant reinforcement in respect to their DA dependence. Thus, while psychostimulant reinforcement in animals is specifically impaired by lesion or blockade of DA transmission, this is not the case for food reinforcement. It has been argued (Berridge and Robinson, 1998) that amphetamine reward is DA independent on the basis of the reported failure of D2 receptor antagonists to affect self-reported measures of amphetamine 'liking' (Brauer et al., 1997). However, brain imaging studies reveal the existence of a high correlation between the increase of extracellular DA (as estimated from the displacement of C11-raclopride binding) in striatal areas and self-reported 'high' (liking, euphoria) following administration of cocaine, methylphenidate (Volkow et al., 2002) and amphetamine (Drevets et al., 2001). Experience of a 'high', following cocaine or methylphenidate (MP), is a function of rate rather than steady-state level of DAT blockade (Volkow et al., 1995, 1997a, 1999a,b,c). Slow blockade of DAT following oral drug administration fails to elicit a 'high' due to the failure to increase DA rapidly and to occupy a sufficient proportion of striatal DA receptors within a short time period (Volkow et al., 1997a, 1999a, 2000). Thus, the powerful reinforcing properties of smoked 'crack' seem related to the fact that the pulmonary route provides the most rapid means to block DAT and to increase DA in the striatum.

This lesson might be eventually extended to other drugs of abuse known to depend upon a rapid entry into the brain compartment via smoking, such as nicotine and cannabis.

A relationship that, in the studies of Volkow and colleagues, seems to hold quite consistently, is that between the displacement of C11-raclopride by endogenous DA in

the striatum and self-reported 'highs' (Volkow et al., 1999c; Di Chiara, 2002). This point is of critical importance, since it demonstrates the power of the correlative approach, particularly if one considers that experimental studies utilizing D2–DA receptor blockers have consistently failed to demonstrate a role of DA in psychostimulant liking (Brauer et al., 1997). This correlative evidence can now provide the basis for critically evaluating the reasons for the negative outcome of these experimental studies. One reason for this might be the insufficient degree of blockade of DA–D2 receptors by neuroleptics administered at doses sufficiently low to avoid dysphoria, an effect that could otherwise interfere nonspecifically with self-reported measures of euphoria. Indeed, if dysphoria is an unavoidable consequence of full D2 receptor blockade, it becomes an almost impossible task to demonstrate a role of DA receptors in psychostimulant high by the use of a D2 antagonist. Recently, however, positive results have been reported with a D1 antagonist (Romach et al., 1999).

The relationship demonstrated by PET studies between psychostimulant-induced high/euphoria and an increase of extracellular DA in the striatum, and in particular in its ventral subdivision (Drevets et al., 2001; Volkow et al., 2002) is important for the understanding of the neurobiological substrates of motivated behavior in its normal and abnormal aspects, such as drug addiction. In particular, these observations challenge the view that the euphoria/high (liking, hedonia, etc.) elicited by psychostimulants is independent of DA transmission (Berridge and Robinson, 1998). This assumption, initially referred by its proponents specifically to the hedonic reactions to taste stimuli in rats, has been later extended to euphoria elicited by psychostimulants in humans on the basis of the studies by Brauer et al. (1997). Failure to acknowledge the differences between food and psychostimulant reward led to two opposite views of reward both affected by an overgeneralization bias. Thus, by extending to conventional rewards the role of DA in psychostimulant reward, Wise (1982) came to postulate a role of DA in all rewards (original anhedonia hypothesis). Vice versa, by extending to psychostimulants the negative evidence they obtained on the role of DA in taste reward, Berridge and Robinson (1998) went on to negate a role of DA in any reward. These two opposite views, however, in their generalizing impetus, have failed to recognize the different nature of the reward induced by food/taste and by psychostimulants respectively. Thus, psychostimulant reward is characterized by euphoria, an hedonic state (state-hedonia), while food/taste reward is characterized by hedonic sensory cues (sensory-hedonia). We argue that euphoria is DA dependent while sensory–hedonia is DA independent. This would account for the exquisite sensitivity of psychostimulant reward to the impairment of DA transmission and, conversely, the relative resistance of food/taste reward. We therefore propose that DA-mediated state-hedonia is a basic component of the incentive arousal state induced by psychostimulants. This state would be composed by an hedonic component providing motivational valence to the drug stimulus and an activational component facilitating response expression.

6. IN VIVO, MONITORING OF DOPAMINE FUNCTION: METHODOLOGICAL ISSUES

Studies involving manipulation of DA transmission by drugs or lesions, while essential for providing experimental evidence for a role of DA in behavior, are nonetheless unable to clarify the quantitative and temporal relationship between the activity of DA

transmission and behavior. To this end, correlative evidence obtained by monitoring the activity of DA neurons or the extracellular levels of DA in response to motivational stimuli and during behavior are necessary. These studies, however, are fraught with a number of difficulties related to the specific dopaminergic nature of the signals recorded. Therefore, it is essential that independently established criteria of specificity be applied and satisfied. Unfortunately this approach has not always been followed, which in part explains the large discrepancies existing in the literature over this topic. More importantly, some theories of DA function have utilized as supporting evidence the results of correlative studies where the nature of the recorded signals was not unambiguously characterized.

Recording of the activity of DA neurons in awake, restrained but behaving or freely-moving animals can be achieved by extracellular electrodes. DA neurons can be identified on extracellular recordings on the basis of the shape of their action potential (spike), pattern and rate of their generation (firing activity), conduction velocities upon antidromic activation, site of projection and pharmacological reactivity (Grace and Bunney, 1983). These criteria however have all been rarely applied in a given study. Even if the DA nature of the signals recorded is characterized, some inadequacies intrinsic to the methodology remain. Thus, extracellular recording of DA neuron activity suffers from a sampling bias related to the fact that only a fraction of DA neurons is spontaneously active but only spontaneously active neurons can be detected by extracellular recording and utilized for further study. It is likely that different populations of DA neurons do exist and are characterized by different spontaneous firing activity. Moreover it is likely that the response of DA neurons to stimuli is dependent upon the basal rate of spontaneus firing.

This situation contrasts with that of another technique currently uitlized to estimate in vivo, DA function, brain microdialysis (Ungherstedt, 1984; Di Chiara, 1990; Westerink, 1995; Di Chiara et al., 1996). This technique involves the unambiguous sampling of DA present in the extracellular compartment recovered by dialysis perfusion throughout implanted probes. In this case, basal as well as stimulus-induced changes are the expression of the activity of the whole population of DA neurons terminating in the implanted area, including those otherwise silent. These advantages of microdialysis are however tempered by its low time resolution (minutes), local tissue damage and relative distance from releasing sites due to the large probe size (> 0.2 mm diameter).

A third methodological approach to the estimation of the DA function in vivo, and to its relation to behavior, is electrochemistry (voltammetry). In this case DA is detected through the current generated by its oxidation (Kissinger et al., 1973; Wightman and Robinson, 2002; Robinson et al., 2003). This method has the advantage of high time-resolution over microdialysis (< 1 s), low tissue damage and short distance from release sites due to the small size of the probes (0.005–0.030 mm), although some chrono-amperometric techniques utilize electrodes made up of three 0.030 mm carbon fibers (total diameter: 0.100 mm). A major disadvantage of electrochemical techniques is the lack of specificity due to the fact that the signal measured is not specific of a given chemical species but is common to many species some of which coexist in the extracellular fluid of a given area. Various methods have been devised to correct this inadequacy. In chronoamperometry the ratio between the oxidation and the reduction current for DA is different from that of two main interfering substances as ascorbic acid and DOPAC (Gratton et al., 1989; Gerhardt and Hoffman, 2001). These differences however are relatively small (DA, 0.7; DOPAC, 1.0) and in some studies the redox ratios recorded

during behavior are broad enough to overlap the differences in redox ratio between DA and nonDA species (Gratton and Wise, 1994; Kiyatkin and Gratton, 1994).

Another problem with this methodology is the fact that background current must be subtracted to obtain the specific contribution of the species of interest to the overall signal, in chronoamperometry this can be made only at the beginning of the experiment. As a result, any change in background current taking place during the experiment cannot be distinguished from that due to the intended species. Changes in background current are known to take place in voltammetry as a result of changes in oxygen tension and pH induced by changes in blood flow due in turn to changes in synaptic activity (Gerhardt and Hoffmann, 2001; Venton et al., 2003). Therefore, in chronoamperometric studies the recorded changes in voltammetric signal will incorporate changes in background current.

One way that has been pursued in an attempt to reduce the influence of interfering compounds is by coating voltammetric electrodes with substances that prevent their contact (ion exchange resins, stearate) (Gerhardt et al., 1984; Blaha and Phillips, 1996). However the selectivity of these electrodes has been questioned (Whightman and Robinson, 2002).

Fast-scan cyclic voltammetry (Stamford et al., 1984; Millar et al., 1985) provides a way out of this problem since, by scanning the voltammetric signal every 100 ms, it enables the online subtraction of changes in background current, including those related to changes in pH and O_2 due to changes in blood flow (Venton et al., 2003). Continous scanning of the voltammetric signal also provides a means to test the nature of the current being measured and to assign it at least to a given chemical class. Thus, fast-scan cyclic voltammetry allows a distinction between DA and other substances oxidized at the same potential, such as ascorbate, DOPAC and uric acid but fails to distinguish it from another catecholamine such as noradrenaline due to the similarity of their voltammogram (Whightman and Robinson, 2002). Thus, this technique is not applicable to study DA transmission in areas where DA and noradrenaline coexist. This might explain the preference of voltammetric studies for the NAc core, an area devoid of noradrenergic innervation, over the noradrenaline-rich NAc shell, which in turn contrasts with the wealth of microdialysis studies in this NAc subdivision. An important characteristic of fast-scan cyclic voltammetry is the fact that it is a differential tecnique, that is, it detects changes between two temporally adjacent (100 ms) scans. This means that only changes with half-time around 100 ms can be detected. Changes taking place over times longer than a second, including changes in steady-state levels of DA, will not be detected. Therefore failure to detect basal DA levels by fast cyclic voltammetry is not simply due to its insufficient sensitivity in detecting DA, but to the differential way it estimates voltammetric signals.

These considerations highlight the fact that microdialysis and voltammetry, particularly fast-scan cyclic voltammetry, estimate two quite different aspects of DA transmission characterized by different temporal and spatial constants. Microdialysis estimates steady-state levels of extracellular DA and changes in these levels taking place on a minute scale away from DA release sites. Fast-scan voltammetry estimates changes in DA-like signals taking place on a subsecond scale near DA release sites.

On this basis we conclude, in agreement with Whightman and Robinson (2002), that microdialysis and voltammetry, rather than alternatives, are complementary techniques for monitoring DA transmission in vivo.

An overview of the methods available for in vivo monitoring would not be complete without mentioning brain imaging techniques (Volkow et al., 1994, 2002;

Drevets et al., 2001). These techniques have been applied to the estimation of extracellular DA as measured indirectly from the availability of D2 receptors to the speciifc D2 ligand C11 raclopride.

6.1. STIMULUS RESPONSE PROPERTIES OF DOPAMINE NEURONS AND DOPAMINE TRANSMISSION

Various studies show that the DA neuron activity and the DA transmission is activated in relation to the presentation of motivational stimuli or to the expression of specific phases of motivated behavior.

Electrophysiological studies of presumed DA units in the monkey mesencephalon have revealed that DA neurons respond by a burst of spikes to the unpredicted occurrence of primary food stimuli and of stimuli conditioned to them (Schultz et al., 1993; Schultz, 1998). Conversely, unpredicted omission of reward results in the inhibition of DA actitvity. Predictability of reward occurrence or omission results in loss of the ability to activate or inhibit the activity of DA neurons. On this basis it has been argued that DA neurons code for errors in the prediction of reward occurrence (Schultz, 1998). These properties of DA neurons conform to what formal learning theory would predict for a neural substrate of associative learning. From this, it has been inferred that phasic DA serves the function of a teaching signal in learning processes related to the ability to predict reward occurrence in the context of motivated behavior (Schultz, 1998). These observations, on the other hand, are not consistent with the original anhedonia hypothesis.

Microdialysis studies on the effect of feeding and food-related stimuli on the extracellular DA have shown that feeding releases DA under selected condiditons and in specific brain areas.

Thus, the response of DA transmission to feeding depends on food palatability, taste novelty, predictability, deprivation state (hunger) and terminal DA area. Early studies showing an increase of DA in dialysates from the NAc (Hernandez and Hoebel, 1988; Radhakishun et al., 1988; McCullough and Salamone, 1992; Yoshida et al., 1992; Inoue et al., 1993; Westerink et al., 1994; Wilson et al., 1995) or from the caudate-putamen (Church et al., 1987) utilized rats deprived of food for 24–36 h or maintained at 80% of their free-feeding weight by food restriction. Some studies utilized a scheduled presentation of food in food-restricted rats in free-feeding (Church et al., 1987; McCullough and Salamone, 1992b; Inoue et al., 1993) or in operant conditions (Hernandez and Hoebel, 1988; McCullough et al., 1993b). In this case, the increase in DA was related to motor activity related to instrumental responding or to schedule-induced behavior rather than to the amount of food consumed (McCullough and Salamone, 1992; McCullough et al., 1993b). In the undeprived rats, however, Cenci et al. (1992) found an increase in the PFCX but not in the NAc. Wilson et al. (1995) later showed that food deprivation increases the responsiveness of NAc DA to feeding. Martel and Fantino (1996a) obtained a robust release of DA from the NAc shell following feeding of palatable food but not familiar, less palatable chow. Even in operant conditions and in food-restricted rats however, Datla et al. (2000) failed to observe an increase of DA in the NAc core after intraoral sucrose. The stimulatory response of DA transmission to food is related to its taste rather than to its postconsummatory effects. These responses differ among different terminal DA areas in relation to their motivational valence (appetitive/ aversive) and value (palatability), novelty and predictability (Bassareo and

Di Chiara, 1997, 1999a,b; Bassareo et al., 2002). Primary taste stimuli increased DA in the NAc core and in the PFCX without delay and independently from their positive (appetitive) or negative (aversive) valence. In contrast, aversive and appetitive stimuli differentially affected NAc shell DA. Thus, NAc shell DA rapidly increased in response to an appetitive unfamiliar taste (sweet chocolate, Fonzies) but was unaffected by a 10 min application of aversive tastes (quinine; saturated NaCl solutions) (Bassareo and Di Chiara, 1997, 1999a,b; Bassareo et al., 2002). Shorter-lasting (5 min, 1 ml) application of aversive stimuli either gustatory (quinine) or olfactory (red fox urine), elicited a delayed and transitory activation of DA transmission in the NAc shell (Bassareo et al., 2002).

Although eventually necessary, positive valence is not sufficient for short-latency activation of DA transmission in the NAc shell by motivational stimuli. Thus, in addition to positive valence relative novelty is also necessary for stimulation of DA transmission (Bassareo and Di Chiara, 1997, 1999a,b; Bassareo et al., 2002). This might explain why sucrose (20%), while no less effective in eliciting hedonic taste reactions than sweet chocolate, fails to stimulate DA transmission in the NAc shell (Bassareo et al., 2002). Furthermore, stimulation of DA transmission habituates after a single exposure to a palatable food in the NAc shell but not in the prefrontal cortex or in the NAc core (Bassareo and Di Chiara, 1997, 1999a,b; Bassareo et al., 2002) (Fig. 6). In the rat, even a mild degree of food deprivation is sufficient to abolish habituation of DA activation in response to palatable food (Bassareo and Di Chiara, 1999b), an observation that might account for the failure of DA neurons to undergo habituation in food-restricted monkeys (Schultz et al., 1993; Schultz, 1998). Habituation of the DA response to intraoral sweet chocolate is not associated with a reduction in hedonic taste reactions (Bassareo et al., 2002) indicating that habituation is unrelated to satiety-induced hedonic devaluation (Rolls et al., 1981; Rolls and Rolls, 1997; Ahn and Phillips, 1999, 2003). Recently Gambarana et al. (2003) have also shown a differential adaptation of DA responsiveness in the PFCX and in the NAc after feeding of vanilla pellets. The rapid habituation of DA responsiveness to food might explain the failure of food reinforcement per se to result in DA release in the NAc in trained undeprived subjects (e.g. Wilson et al., 1995). Close analysis of a study by Martel and Fantino (1996b) is particularly instructive of the pitfalls involved in the peculiar properties of the DA response to food in the NAc shell. In their study Martel and Fantino (1996b) intended to test the role of the amount of food eaten in the responsiveness of NAc shell DA to palatable food. Rats were tested on three consecutive daily microdialysis sessions: on the first day, food was made available ad lib, on the second, was available on a limited amount while on the third, no food was available. As stated by the authors this fixed sequence was adopted of the basis of a previous study (Martel and Fantino, 1996a) showing that the response of DA to palatable food (short cakes), regular chow and no food was independent of the order of presentation. However, as we have shown, habituation is a taste-selective phenomenon (Bassareo and Di Chiara, 1999b). Thus, while chow and shortcakes feeding do not reciprocally interact as a result of habituation, repeated shortcake presentation does. As a smaller amount of short cakes was presented on the second daily session, i.e. 24 h after the first one, when habituation is still fully active, habituation rather than the amount of food could have been the cause of the lower DA response obtained on the second feeding session (Martel and Fantino, 1996b). Based on these observations, the authors rejected their earlier conclusion (Martel and Fantino, 1996a) that the larger DA response to shortcakes over regular chow was due to their higher palatability and attributed their observations to the larger amount of shortcakes eaten over chow (Martel and

Fantino, 1996b). It is likely, however, that both palatability and relative novelty rather than the amount of food eaten were the factors that made shortcakes more effective than regular chow in raising dialysate DA in the NAc shell.

Novelty being a prerequisite for the stimulation of DA release in the NAc shell but not for behavioral hedonic reactions, release of DA in this area is likely to be a consequence rather than the cause of the appetitive properties of taste stimuli, consistent with the idea that taste-hedonia does not depend on DA (Berridge and Robinson, 1998). These observations, however, leave open the issue of a role of DA in state-hedonia (euphoria, eutimia) as distinct from stimulus-bound (e.g. taste) hedonia.

The properties of DA neurons as deduced from microdialysis studies are consistent with the possibility that DA plays different roles in behavior in relation to specific brain areas. The response properties of DA in the NAc shell are consistent with a role in Pavlovian incentive learning (Bassareo and Di Chiara, 1997, 1999a,b; Bassareo et al., 2002). Thus, release of DA in the NAc shell by unfamiliar and unpredicted primary appetitive stimuli (rewards) might serve to associate the sensory properties of the rewarding stimulus with its biological outcome. This mechanism might be, in the case of DA in the NAc shell, related to feeding behavior and responding to unfamiliar, palatable tastes. Thus, release of DA in the NAc by unfamiliar palatable food might serve to associate food taste to its postingestive consequences. In this way, depending on its outcome, the same taste can be accepted or rejected on a further encounter. Instead, the properties of DA transmission in the NAc core and in the prefrontal cortex are more consistent with a role in the expression of motivation, in agreement with the notion of the NAc as an interface between motivation and action (Mogenson et al., 1980).

6.1.1. Dopamine transmission and aversive stimulation

Extracellular recording of presumed DA neurons in the monkey showed that DA neurons are relatively unresponsive to aversive stimuli (Mirenowicz and Schultz, 1996). These observations contrast with those of other studies showing that primary aversive stimuli stimulate the firing of DA units in cats and in rats (Horvitz et al., 1997; Horvitz, 2000) and increase extracellular DA in various terminal DA areas (Abercrombie et al., 1989; Imperato et al., 1989; McCullough et al., 1993a; Kalivas and Duffy, 1995; Bassareo et al., 1996). On the basis of these results it has been hypothesized that DA plays a role not only in appetitive but also in aversive motivation (Horvitz, 2000; Salamone, 1994). These discrepancies could be due to the different nature (e.g. direct versus indirect) of the electrophysiological response as compared to the neurochemical one or to the nature of the unit recorded in the mesencephalon. In relation to this it has been recently reported that VTA neurons activated by aversive stimuli are tyrosine hydroxylase negative i.e. nonDA in nature (Ungless et al., 2004). The view that unconditioned aversive stimuli activate DA transmission, however, fails to take into account the existence of major differences in the responsiveness of the different subdivisions of the DA system to motivational stimuli. Thus, while the response of NAc core and PFCX DA to the intraoral infusion of quinine or saturated NaCl solutions corresponds to that of the current view, the response of the NAc shell is more complex (Bassareo et al., 2002). In the NAc shell the increases in DA function reported by the literature have been obtained under experimental procedures or conditions, such as postmortem estimation of DOPAC/DA ratio (Deutch and Cameron, 1992), long sampling interval in microdialysis studies (20 min or more) or long-lasting (10–20 min) exposures to the aversive stimulus (Kalivas and Duffy, 1995),

that do not allow a precise estimation of the time relationship between the changes in DA function and the aversive properties of the stimulus. Under appropriate conditions, such as application of discrete, short-lasting stimuli and shorter microdialysis sampling time, the increase of NAc shell DA by aversive stimuli does not appear directly related to the aversive stimulus (Bassareo et al., 2002). The delayed nature of the increase of DA in the NAc shell after short-lasting (5 min) taste stimuli and the failure to occur after longer lasting applications suggests that this effect is not the direct consequence of the aversive properties of the stimulus; rather, it might be the effect of the positive state associated with the recovery from the aversive state induced by the stimulus (Bassareo et al., 2002).

What is other than the direct effect of aversive stimuli on NAc shell DA? We have reported that short-lasting (5 min) tail pinch induces an immediate reduction of DA in the NAc shell (Di Chiara et al., 1999). Moreover, in a CTA paradigm, intraoral saccharin increases dialysate DA in the NAc before pairing with an aversive state while decreasing it thereafter (Mark et al., 1991). Intraoral application of quinine (5–10 mM) for 5 min significantly reduced DA in the NAc shell in the first sample (Bassareo et al., 2002). These observations suggest that a phasic, short-lasting inhibition is the immediate effect of aversive stimuli on DA transmission in the NAc shell. Recent electrophysiological studies performed in the rat have found that aversive stimuli (tail pinch) consistently inhibit DA neurons of the VTA (Ungless et al., 2004), thus supporting the observations made with microdialysis (Di Chiara et al., 1999); neurons excited by aversive stimuli turned out to be nondopaminergic. Electrophysiological changes, however, do not always result in a significant change in dialysate DA due to their short-lasting nature (Bassareo et al., 2002).

These observations contradict the widely held belief that DA transmission is activated by motivational stimuli in a uniform fashion across different terminal DA areas in relation to their motivational impact independendently from its motivational valence (Salamone, 1994; Horvitz, 2000). This notion, perhaps appropriate for the NAc core and PFCX, does not apply to the NAc shell where the responsiveness of DA transmission is critically dependent upon additional properties of the stimulus, namely its novelty and motivational valence.

6.1.2. Dopamine release in the n.accumbens by conditioned stimuli

The issue of the ability of conditioned stimuli to release DA in the NAc is debated. Existing discrepancies might be related to a number of variables including the positive or negative valence of the stimulus, the specific terminal area where DA is monitored, the contingent versus noncontingent modality of presentation of the CS and its discrete (cue) versus contextual nature.

As to aversive stimuli, Mark et al. (1991) initially reported that NAc DA release is decreased by presentation of a gustatory stimulus (saccharin) conditioned to visceral malaise (i.p. lithium) in a CTA paradigm but no distinction was made between shell and core. Stimulation of NAc DA transmission by a contextual but not by a discrete stimulus (tone) conditioned to an electric shock was reported by Saulskaia and Marsden (1995). On the other hand, Young et al. (1993) and Wilkinson et al. (1998) reported that discrete stimuli (tone or light) conditioned to footshock stimulate DA release in the NAc. Procedural differences might account for the different results of these studies from that of Saulskaia and Marsden (1995). More recent studies have reported a stimulatory DA response to an aversively conditioned discrete stimulus (tone) in the NAc shell but not

in the core (Murphy et al., 2000; Pezze et al., 2001). No significant changes were observed in the NAc shell in response to an aversively conditioned context (Pezze et al., 2001).

As to appetitive stimuli, Datla et al. (2002) have reported that a simple visual stimulus (illumination of the magazine light) conditioned to sucrose fails to stimulate the release of DA in the NAc. However, if a novel tone is introduced preceding the light–sucrose association, sucrose strongly stimulates the DA release in the NAc and, after 15 of these pairings, presentation of the tone followed by the light CS strongly stimulates DA release in the NAc (Datla et al., 2002). Presentation of the tone alone increases DA only slightly. Similar results have been obtained by the same group in aversive conditioning (Young et al., 1993) and in sensory conditioning (Young et al., 1998). These observations are reminiscent of the observation that complex, rather than simple, cues are necessary for the reinstatement of instrumental responding for cocaine (see Shalev et al., 2002 for review). These results suggest that stimulus salience resulting from its specific sensory modality (acoustic), complexity (multimodal), novelty and unpredictability is an important factor in the ability of conditioned stimuli to activate DA transmission.

The above studies do not differentiate between shell and core subdivisions. Such distinction, however, might be important. Thus, release of DA in response to food-conditioned olfactory stimuli has been observed in the NAc core and in the PFCX but not in the NAc shell by Bassareo and Di Chiara (1997, 1999a). On this basis it was concluded that NAc core and PFCX DA but not NAc shell DA responds to Pavlovian appetitive stimuli. This might apply to the observation of Mark et al. (1991) that taste stimuli conditioned to caloric foods increase DA release in the NAc. Recently however, Cheng et al. (2003) have reported that an acoustic stimulus (10 s white noise) paired with food reward acquire incentive properties in the form of nose pokes into the food tray as well as DA releasing properties in both the shell and the core of the NAc. Under the same paradigm no conditioned release of DA was obtained in the PFCX in response to a discrete auditory CS (Mingote et al., 2004). These results are different from those of Bassareo and Di Chiara (1997, 1999a) who reported release of DA in the NAc core and PFCX but not in the NAc shell after presentation of an appetitive CS. Many experimental differences might account for the different results obtained in these studies, among these, differences in sensory modality, nature and duration of the CS (5 min presentation of a complex, mainly olfactory versus 10 s presentation of an acoustic CS), in the number of pairings (three sessions with a single pairing per session versus three sessions with six pairings per session) and finally, differences in motivational state (food ad lib versus food restriction). These differences point to the possibility that NAc shell and PFCX DA differentially code for food-conditioned stimuli in relation to the specific experimental conditions.

As to drug-conditioned stimuli, Fontana et al. (1993) and Duvauchelle et al. (2001), in contrast to previous negative results of Brown and Fibiger (1992), have reported an increase of NAc DA in response to contextual, cocaine-conditioned stimuli. In none of these studies was a distinction between NAc shell and core was made. Such a comparison was investigated by Ito et al. (2000) in rats self-administering cocaine on a second order schedule. In this study, noncontingent presentation of the discrete CS resulted in the release of DA in the NAc core but not in the shell. Contingent presentation of the conditioned reinforcer was ineffective. Recently however, Bradberry and Rubino (2004) reported that in monkeys noncontingent presentation of a complex olfactory-visual-auditory cue predictive of the availability of cocaine for i.v. self-administration and effective in eliciting anticipatory bar-pressing, fails to stimulate DA release in striatal

as well a prefrontal (orbitofrontal and anterior cingulate) cortical areas. This observation in turn is at variance with the studies in rats by Weiss et al. (2000) who observed release of DA in the NAc upon presentation of discriminative cues previously associated to i.v. cocaine and effective in reinstating extiguished responding. Various experimental differences (species, extinction schedule, discriminative nature of the stimuli) can account for these discrepancies. Thus, the ability of drug-conditioned cues as to their ability to stimulate DA release in microdialysis studies is a complex function of the specific properties of the stimulus and of terminal DA area.

Recently, we have completed a series of studies comparing the effect of Pavlovian stimuli conditioned to morphine and food on DA relase in the NAc shell, NAc core and PFCX (Bassareo et al., in preparation). A more detailed account of these studies will be given in the Section *Drug-induced stimulation of DA transmission and abnormal Pavlovian incentive learning*. Here it will suffice to say that while drug-conditioned stimuli elicit a sustained release of DA in the NAc shell but not in the NAc core, food-conditioned stimuli release DA in the NAc core but not in the shell (Figs. 6–8).

Presentation of a response contingent CS does not result in release of DA in the NAc as estimated by microdialysis (Bradberry et al., 2000; Neisewander et al., 1996). Similarly, Ito et al. (2000) did not observe any change of dialysate DA in the NAc shell or in the NAc core in relation to bar-pressing for a cocaine-conditioned stimulus.

Recently, a number of studies have applied the technique of fast scanning cyclic voltammetry to the study of the DA responses to CS in the context of instrumental responding for intraoral sucrose and for i.v. cocaine. In these studies voltammetric signals attributed to DA on the basis of their voltammogram were recorded every 100 ms from the NAc core. In rats trained to bar press for intraoral sucrose, noncontingent presentation of the cue light+retractable lever signaling sucrose availability resulted in a subsecond increase of the DA signal which peaked around the time of bar-pressing. In some rats the cue elicited a late transient starting before the response and peaking at its start (Roitman et al., 2004). In rats self-administering cocaine i.v. two small increases preceding and a large increase immediately following the start of bar-pressing were recorded (Phillips et al., 2003). The two preresponse changes seem related respectively to the arousal preceding approach and to actual approach of the lever. The large postresponse change is related to the occurrence of a 20 s audiovisual stimulus conditioned to cocaine infusion and, accordingly, can be evoked also by noncontingent presentation of the stimulus alone (Phillips et al., 2003). The authors interpret these observations to mean that phasic DA promotes responding for cocaine and in support of this show that electrical stimulation of the VTA induces responding for cocaine. However, the scale of DA changes after this manipulation is more than one order of magnitude higher than preresponse changes and therefore not comparable with them. A more parsimonious explanation for both pre- and postresponse changes is that they are the substrate of the incentive arousal state induced by Pavlovian stimuli and as such act to facilitate instrumental action. In these studies the changes in DA signal in rats self-administering sucrose are different from those occurring in rats self-injecting cocaine. In sucrose self-administration, a single large change occurs which precedes the response and peaks around its start (Roitman et al., 2004). In cocaine self-administration, while changes preceding the response are very small, a large postresponse change occurs related to the cues that operate following the start of infusion (Phillips et al., 2003). These differences have been explained by a differential reward-predictive value of response contingent stimuli in cocaine as compared to sucrose

reinforcement related in turn to the fact that, following responding, while cocaine reward is delayed, sucrose reward is immediate (Roitman et al., 2004).

These studies show that phasic DA transmission is activated by Pavlovian stimuli in a manner consistent with its postulated role in incentive motivation.

6.2. DRUG MOTIVATED BEHAVIOR: CORRELATIVE STUDIES

On the basis of the mechanism of action of drug rewards on DA transmission, it is useful to distinguish them into two broad categories: psychostimulants, including cocaine and amphetamine-like drugs (amphetamine, methamphetamine, phencyclidine, ecstasy, khat) and nonpsychostimulants (including opiates, cannabinoids, ethanol, barbiturates, GHB and nicotine). The psychostimulants increase the concentration of DA in the extracellular compartement (EC) by acting directly on DA mechanisms either by blocking DA reuptake (cocaine) or promoting the carrier-mediated outflow of DA from terminals (see Di Chiara, 1995, for review). The psychostimulants utilize the DA as a primary mechanism of their reinforcing actions, other mechanisms (e.g. blockade of the NA or 5HT carrier by amphetamine and cocaine) being ancillary and nonessential for reinforcement under normal conditions. Nonpsychostimulant drugs act primarily on nonDA neurons or on nonDA mechanisms and increase DA concentrations in the EC secondary to these nonDA actions. Nonpsychostimulant drugs increase EC DA by increasing the exocitotoxic release of DA either by stimulating the firing activity of DA neurons or increasing the efficiency of the release process or both (see Di Chiara, 1995, for review). Psychostimulant drugs actually reduce exocitotoxic release of DA as a result of activation of DA autoreceptors by endogenous DA.

6.2.1. Microdialysis studies

Transcerebral brain microdialysis studies of the effects of addictive drugs on DA transmission in the dorsal caudate-putamen and in the NAc have shown that not only psychostimulants like cocaine (Kuczenski and Segal, 1992) and amphetamine (Carboni et al., 1989) but also narcotic analgetics (Di Chiara and Imperato, 1988a), nicotine (Imperato et al., 1986), ethanol (Imperato and Di Chiara, 1986) and phencyclidine (Carboni et al., 1989) stimulate DA transmission preferentially in the NAc, as compared with the dorsal caudate-putamen. If one considers the NAc and the dorsolateral caudate-putamen as representatives of the ventral and of the dorsal striatum respectively, drugs of abuse appear to exert a preferential action on in vivo DA transmission in the ventral as compared with the dorsal striatum (Di Chiara and Imperato, 1988b). Failure of some studies to observe differences in the DA stimulant effects of amphetamine between the NAc and the dorsal caudate-putamen (Robinson and Camp, 1990) was explained as due to differences in the placement of microdialysis probes in the dorso-ventral dimension (Di Chiara, 1991). Recently, Drevets et al. (2001) have studied by PET in humans the in vivo binding of C11 raclopride as a reciprocal index of the level of DA in the EC after amphetamine administration at different horizontal levels in the striatum and its correlation with self-reported measures of euphoria. Amphetamine reduced ligand binding to a greater extent in the ventral striatum than in the dorsally located caudate-putamen and this effect was highly correlated with euphoria measures in ventral but not in dorsal striatal areas. Since the analysis of Drevets et al. (2001) was performed in the horizontal plane, its results can be directly compared with those obtained in the rat by

Di Chiara and Imperato (1988) and by Carboni et al. (1989) with horizontal microdialysis probes and are consistent with the existence of a dorsoventral gradient within the striatum in the responsiveness of DA transmission to drugs of abuse (Di Chiara, 1989). Further studies of the effect of amphetamine on extracellular DA with concentric microdialysis probes vertically placed at different mediolateral levels in the NAc showed significant differences between the dorsal caudate-putamen and the medial but not the lateral part of the NAc (Di Chiara et al., 1983), suggesting the existence of a mediolateral heterogeneity in the responsiveness of striatal DA to amphetamine. Further evidence along this line is provided by studies in Rhesus monkeys self-administering unit doses of 0.5 mg/kg of cocaine i.v. showing that cocaine increased DA more effectively in the ventromedial striatum as compared with the central and dorsal striatum and in the medial as compared with the central and lateral striatum (Bradberry et al., 2000). This evidence is particularly important since it refers to response-contingent administration of cocaine in a nonhuman primate.

These studies indicate the existence within the striatum of a gradient of increasing responsiveness to psychostimulants directed ventromedially that corresponds to the notion of a preferential action in the ventral striatum.

The mechanism by which drugs of abuse stimulate DA transmission in the striatum is different depending on the drug class they belong to. The preferential stimulation of terminal DA transmission in the NAc is associated in the case of nonpsychostimulant drugs (narcotic analgetics, ethanol, nicotine) to a preferential stimulation of the firing activity of DA neurons of the ventral tegmental area, known to innervate the ventral striatum, as compared with pars compacta neurons, known to project mainly to the dorsal striatum (Mathews and German, 1984; Gessa et al., 1985; Mereu et al., 1987). As to the preferential effect of psychostimulants in the NAc, a lower efficiency of DA reuptake in the NAc as compared with the dorsal striatum has been suggested as the mechanism (Cass et al., 1992). More recently two factors have been found to be inversely correlated to the ability of psychostimulants to increase extracellular DA, the amount of DA released per pulse after a 20 Hz stimulation and the rate of DA reuptake (Wu et al., 2001). The NAc shows a reduced ability to release and the uptake of DA compared with the caudate-putamen (Wu et al., 2001). Similar differences have been reported for the NAc shell compared with the core (Jones et al., 1996). These differences are consistent with the lower density of the DAT, as estimated by ligand autoradiography, in the NAc compared with the caudate-putamen (Marshall et al., 1990) and in the NAc shell compared with the core (Jones et al., 1996).

6.2.1.1. Localization of the dopamine stimulant effects of drugs within the striatum

Histochemical and connectional studies have distinguished in the NAc a ventromedial 'shell' and a dorsolateral 'core' (Alheid and Heimer, 1988; Heimer et al., 1991). On the basis of their input–output relationships, these two subdivisions have been assigned a different functional significance, the 'core' being involved in motor functions and the 'shell' being involved in emotion as a transition area of the extended amygdala (Alheid and Heimer, 1988; Heimer et al., 1991). In order to verify the possibility of shell/core differences in responsiveness of DA to drugs of abuse, rats were implanted with intravenous catheters and with concentric microdialysis probes aimed at the NAc 'core' of one side and at the 'shell' of the other side and the changes in DA transmission were studied after various drugs given i.v. at unitary doses known from the literature to

maintain self-administration behavior in the rat. Calbindin immunohistochemistry was utilized to distinguish 'shell' from 'core' in the histological verification of probe location (Pontieri et al., 1995, 1996; Tanda et al., 1997). These studies showed that nonpsychostimulant drugs (including morphine, heroin, nicotine and δ9-tetrahydrocannabinol), at each of the two doses tested, increased dialysate DA selectively in the NAc shell. Cocaine showed a selective effect in the shell at lower doses and a preferential one at higher doses. Amphetamine showed a preferential effect in the shell at lower doses but at higher doses the effect was similar in the shell and in the core. Similar observations were made for morphine and amphetamine given subcutaneously and for cocaine given intraperitoneally (Cadoni and Di Chiara, 1999, 2000; Cadoni et al., 2000). More recently a preferential effect of amphetamine in the anterior shell has been reported after local intracerebral infusion (Heidbreder and Feldon, 1998). Similar findings have been reported by Barrot et al. (1999) for morphine and cocaine. In parallel studies with 2-deoxyglucose autoradiography it was also shown that nicotine, morphine, cocaine and amphetamine activate at low doses energy metabolism selectively in the NAc shell, indicating that stimulation of DA transmission in this area by drugs of abuse increases the activity of intrinsic and afferent neural input (Pontieri et al., 1994, 1996; Orzi et al., 1996).

6.2.1.2. Specificity of the dopamine stimulant properties of addictive drugs

The property of addictive drugs to stimulate DA transmission in the NAc shell is specific in many respects. Thus, caffeine, a drug with psychostimulant and rewarding properties but devoid of addictive properties dose-dependently increases dialysate DA in the prefrontal cortex but is ineffective on DA transmission in the NAc shell or core (Acquas et al., 2002). The effect of caffeine on DA in the prefrontal cortex might be secondary to its psychostimulant properties, which in turn might be the result of blockade of A2 and A1 adenosine receptors in limbic areas. Given the lack of addictive properties of caffeine (American Psychiatric Association, 1994), its failure to stimulate DA transmission in the NAc shell is consistent with a role of NAc shell DA in the addictive properties of drugs. Apart from psychostimulants, addictive drugs do not increase DA transmission in the prefrontal cortex. Thus, nonpsychostimulant drugs including morphine, ethanol and nicotine, at doses which fully stimulate DA transmission in the NAc shell, do not increase DA transmission in the medial prefrontal cortex where mesocortical DA neurons terminate (Bassareo et al., 1996). Cocaine and amphetamine, however, increase dialysate DA in the prefrontal cortex even more effectively than in the NAc shell (Tanda et al., 1997). The increase in extracellular DA in the prefrontal cortex induced by cocaine and amphetamine, however, is not due to an action on the DA carrier (as in the NAc) but to the blockade of the noradrenaline (NA) carrier, as shown in vivo by the concurrent increase of NA in the prefrontal cortex (Tanda et al., 1997). GBR 12909, a blocker of the DA carrier devoid of action on the NA carrier, while fully increasing DA in the NAc, is ineffective in raising extracellular DA in the prefrontal cortex. Moreover, under selective blockade of the NA carrier by desipramine through reverse dialysis, cocaine fails to increase DA in the prefrontal cortex (Tanda et al., 1997). These observations are explained by the 100-times difference in the ratio of NA terminals to DA terminals in the prefrontal cortex as compared with the NAc (Palkovits 1979) and by the high efficiency (four times more than NA itself) of the NA carrier as a transporter of DA (Raiteri et al., 1977). Therefore, in the prefrontal cortex NA terminals provide a means for the clearance of DA from the extracellular space that is more efficient than that provided by DA terminals.

Although the role of the increase of DA in the prefrontal cortex for the addictive properties of cocaine and amphetamine is obscure, it is unlikely to be a major one given the lack of addictive liability or psychostimulant properties of antidepressants, that increase DA in the PFCX but not in the NAc (Tanda et al., 1994). Finally, a number of aversive-anxiogenic drugs (e.g. picrotoxin, pentylenetetrazol, beta-carbolines) stimulate in vivo DA transmission in the medial prefrontal cortex but fail to affect DA transmission in the NAc shell (Bassareo et al., 1996). These observations indicate that the property of stimulating DA transmission in the NAc shell is not secondary to generic motivational stimulus properties or to psychostimulant properties of addictive drugs.

Recent microdialysis studies show that DA transmission in the bed nucleus of stria terminalis (BNST) is activated by drugs of abuse as efficiently as in the NAc shell (Carboni et al., 2000). The role of this effect in the reinforcing and addictive properties of drugs is unknown. The central amygdala is an area related to the BNST. Local infusion of a D1 antagonist (SCH 23390) in this area impairs cocaine self-administration (Caine et al., 1995).

6.2.1.3. Response contingent versus response noncontingent stimulation of dopamine transmission by drug rewards

A criticism that can be raised towards the applicability of the above observations to the case of active drug self-administration is the fact that drug administration was passive i.e. noncontingent upon a response (Dworkin et al., 1992). The most direct way to address this issue is obviously that of monitoring changes in extracellular DA in animals actively self-administering the drug.

Response contingent drug administration, however, necessarily involves the acquisition of a drug response association and therefore a preexposure to the drug itself. Drug preexposure, however, can affect the relative responsiveness of the different subdivisions of the DA system to the drug reducing the shell/core ratio of drug-induced increase of extracellular DA (Cadoni and Di Chiara, 1999, 2000; Cadoni et al., 2000).

Another factor that can affect the responsiveness of the DA systems to the drug, particularly in the case of psychostimulants, is food restriction (a practice widely utilized in drug self-administration studies). Finally, a critical factor that is most often disregarded in studies comparing the effects of contingent versus noncontingent drug administration on DA transmission is that of the precise localization of the probe within the NAc. Hemby et al. (1995) reported that heroin increases DA in the NAc in experimenter administered but not in self-administering rats and on this basis concluded that heroin increases DA in the NAc only when administered noncontingently upon a response. However, in contrast with the negative results of Hemby et al. (1995), steady increases of NAc DA around 200–250% has been observed by Wise et al. (1995) during i.v. self-administration of 0.05–0.20 mg/kg unit dose of heroin. After higher unit doses of self-administered heroin (0.40 mg/kg) DA increased to a plateau of 350–400% (Wise et al., 1995). Failure to consistently implant microdialysis probes in the medial NAc (i.e. in the shell) rather than response contingency of drug administration might account for the failure of Hemby et al. (1995, 1999) to observe an increase of DA in rats self-administering heroin. Thus, inspection of the location of the microdialysis probes in the studies by Hemby et al. (1995, 1999) shows that they were located in the core or at the shell/core border. This possibility is further suggested by the fact that the combined self-administration of cocaine and heroin increased dialysate DA more effectively than cocaine alone (Hemby et al., 1999).

In this case, blockade of DA reuptake by cocaine might have allowed DA diffusing from the adjacent shell to be recovered by the microdialysis probe located in the core.

Hurd et al. (1989), in the first study on this issue, reported that response noncontingent i.v. administration of cocaine increased dialysate DA in the NAc while response contingent administration was ineffective. In contrast to the above results, various studies from different laboratories have subsequently demonstrated an increase of DA in the NAc of rats (Petitt and Justice, 1989, 1991; Wise et al., 1995; Hemby et al., 1997, 1999; Ito et al., 2000) and in the ventral and dorsal striatum of monkeys self-administering cocaine i.v. (Bradberry et al., 2000).

The reason for the negative results of Hurd et al. (1989) has been tentatively attributed to the fact that results were expressed as absolute DA values rather than normalized to individual basal values and also to the incomplete and erratic stabilization of basal DA (Di Chiara, 1995). Hemby et al. (1997) have compared the effect of response contingent and response noncontingent i.v. administration of cocaine on dialysate DA in the rat NAc. They report a larger increase of DA after contingent administration of cocaine. Thus, opposite to that reported for heroin (Hemby et al., 1995), it was concluded that cocaine is more effective in raising DA in the NAc when administered in a response contingent fashion (Hemby et al., 1995). This conclusion is also consistent with previous observations by Wise et al. (1995) in the NAc. Recent studies by Ranaldi et al. (1999), however, suggest that the conclusions of Hemby et al. (1997), if valid for cocaine, may not be so for psychostimulants in general. In fact, in rats responding for i.v. amphetamine, the increase of DA in the NAc was similar to that observed in yoked controls either naive or experienced to cocaine (Ranaldi et al., 1999). Clearly, more studies are needed to settle this issue.

6.2.1.4. Cocaine increases extracellular dopamine in the nucleus accumbens shell in a response contingent manner

An important question that awaits an adequate answer is if addictive drugs increase preferentially DA in the NAc shell also when administered in a response contingent manner. In an attempt to answer this question, Ito et al. (2000) compared changes in dialysate DA in the NAc shell versus core in rats self-administering cocaine i.v. on a second order schedule but obtained only a tendency for a preferential increase of DA in the shell. Recently we have completed a study (Lecca et al., in preparation) on 10 rats implanted with guide cannulas aimed at the NAc shell of one side and at the NAc core of the other side and with i.v. catheters. Each day a microdialysis probe was inserted on one side and perfusion was started. Rats were connected to the pump and placed in the self-administration cage equipped with two nose-poke holes, one active and the other inactive. Self-administration session was started after 60 min of perfusion. Nose-poke into the active hole would result in i.v.self-administration of cocaine (0.25 mg/kg in 2 s). Rats self-administered cocaine in single daily 1 h sessions on a FR1 schedule for the first 5 days and on a FR5 for the following 10 days. Dialysate DA was monitored for 90 min in 10 min samples taken on alternate days from the NAc shell and core starting on the 1st exposure to the drug. Figure 4 shows the mean of the changes obtained in the NAc shell and in the core. Three way ANOVA showed a significant main effect of area [$F(1,64) = 7.51$, $p < 0.01$) and time [$F(9,576) = 51.45$, $p < 0.01$) and a significant area $\times$ time interaction [$F(9,828) = 3.83$, $p < 0.01$) but no significant interaction of area $\times$ day [$F(14,64) = 0.60$, $p = 0.85$, N.S.) and of area $\times$ day $\times$ time [$F(126,576) = 0.98$, $p = 0.56$, N.S.).

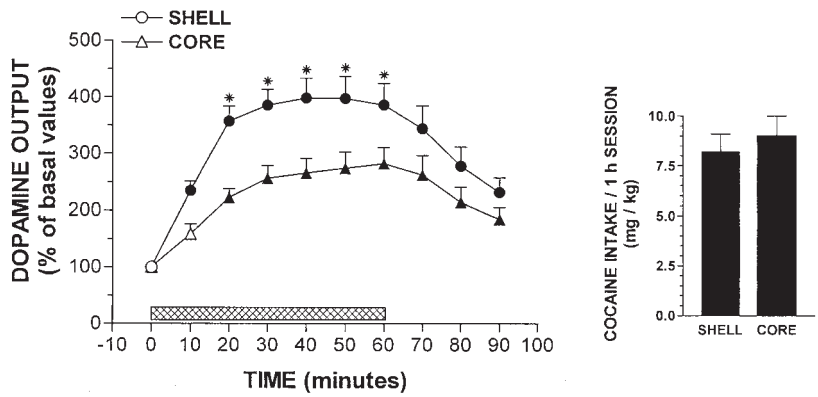

Fig. 4. Mean time-course of DA in the NAc shell and core in 15 daily sessions of cocaine self-administration (left) and mean total cocaine self-administered during each 1h.session (right). Results are means ± SEM of the change of DA concentrations expressed as % of basal. Filled symbols: $p < 0.05$ vs. basal $*p < 0.05$ versus the correspondent value in the core.

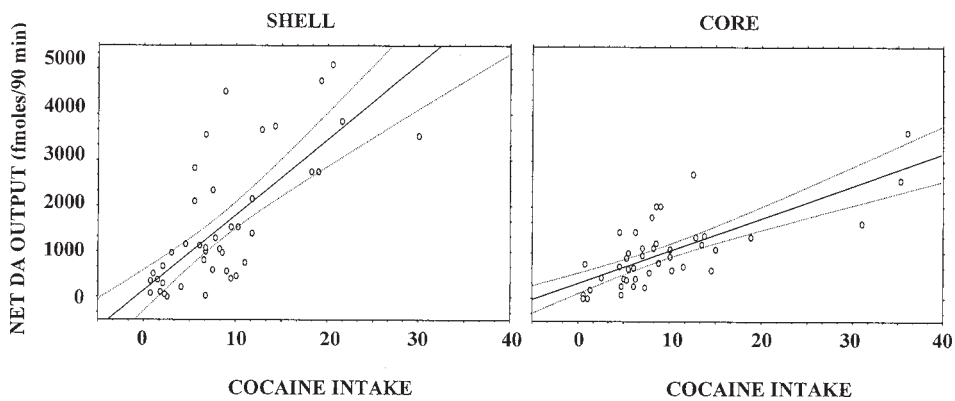

Fig. 5. Regression analysis of the relationship between increase of net DA output in the NAc shell and in the core. Shell: slope = + 165.23; $r = 0.74080$; $p < 0.001$. Core: slope = + 71.049; $r = 0.73946$; $p < 0.001$.

Mean cocaine intake was the same no matter if probes were placed in the NAc shell or in the core (Fig. 4). As shown in Fig. 5, regression analysis of the relationship between increase of DA in the NAc shell and in the core showed a significant correlation in both areas and a significantly steeper slope in the NAc shell compared to the core. These observations extend to response contingent cocaine exposure after the observations reported by us after noncontingent exposure (Pontieri et al., 1995). It is notable that under daily self-administration sessions of cocaine the preferential increase in NAc shell DA was independent from the duration of the exposure to cocaine, indicating that the effect of cocaine on dialysate DA in the NAc does not undergo adaptive changes like tolerance or sensitization.

6.2.2. In vivo monitoring of dopamine transmission by electrochemistry during drug self-administration

Voltammetry has been utilized for monitoring extracellular DA in rats self-administering drugs. These studies have utilized electrodes coated with materials (Nafion, stearate) that drastically reduce the contact with the active electrode surface, and therefore, the contribution to the electrochemical signal of acidic electroactive species as ascorbic acid and DOPAC and oxidized at potentials near those of DA. In this way, a selectivity ratio of 1500 of DA to ascorbate and of 500 of DA to DOPAC is obtained. However, since ascorbic acid is present in the extracellular fluid in concentrations (0.5 mM) 10,000 times those of DA (50 nM) and DOPAC is present in concentrations (0.01mM) 1000 times those of DA, it is unlikely that the goal of selectivity has been achieved. That this might be the case is suggested by the wide redox ratios obtained in vivo. For the above reasons, studies intended to monitor DA transmission in vivo by electrochemistry have been criticized on the grounds of an assumed nonspecificity and unreliability of the electrochemical assay. We have reviewed these studies on the basis of the internal and comparative consistency of the results obtained, i.e. on a posthoc rather than a priori basis and only the conclusions of this analysis will be reported here. To date a number of such studies are available. Most of them have utilized Nafion-coated carbon fiber electrodes (Kiyatkin and Stein, 1993, 1995; Kiyatkin et al., 1993; Gratton and Wise, 1994; Kiyatkin, 1994; Xi et al., 1998) and a series from the same laboratory have utilized stearate-coated fibers (Di Ciano et al., 1995, 1996, 1998a,b, 2001a).

Studies with Nafion-coated electrodes show that, apart from the first heroin self-injection on every session (Kiyatkin et al., 1993) and the first cocaine self-injection on the second and subsequent sessions (Gratton and Wise, 1994), the earliest change associated to drug, food and water self-administration is a phasic, short-lived decrease in the electrochemical signal. This pattern of change does not correspond to the changes in dialysate DA associated to drug self-administration and is also difficult to explain with the known mechanism of action of the drug. In the case of cocaine it has been argued that the reduction in signal is related to a reduction in the firing of DA neurons (Gratton and Wise, 1994; Kiyatkin and Stein, 1995). However, heroin stimulates DA firing activity. One might concede that the change in extracellular DA correspondent to the recorded change in signal might be so small and transient as to be undetectable by 5 or 10 min microdialysis sampling, however, microdialysis studies with 1 min sampling (Wise et al., 1995) as well as chronoamperometric studies with stearate electrodes with 30 s sampling have failed to detect such phasic reductions (Di Ciano et al., 1995).

A recent study by Xi et al. (1998) utilized single carbon fiber instead of multiple fiber electrodes and a fast-scan voltammetry instead of chronoamperometry. An additional difference was that the rats were first trained to acquire stable rates of heroin self-administration and then were implanted with the electrodes. Under these conditions 60% of the rats responded to self-injection of 0.06–0.1 mg/kg of heroin with a monophasic increase, 20% with a biphasic increase/decrease and 20% with a biphasic decrease/increase of the electrochemical signal (Xi et al., 1998). Only monophasic increases were observed with doses of 0.2 mg/kg of heroin. Tonic monophasic increases in signal were observed also by chronoamperometry with stearate-coated electrodes in a comparative study of cocaine and amphetamine self-administration in parallel with microdialyis (Di Ciano et al., 1995). From this study, however, it appears that, although the effect of drug self-injection on dialysate DA and on the tonic voltammetric signal is qualitatively

similar, important quantitative differences are present. Thus, the time to peak for the electrochemical signal in rats self-administering 0.05, 0.10 and 0.20 mg/kg of amphetamine (75 min, 2, 3 h, respectively) is much longer than that of the dialysate DA (30, 30 and 45 min, respectively). As a result of this the changes in electrochemical signal do not reflect as closely as dialysate DA does the pattern of drug self-administration, being slower to rise during the initial loading phase and slower to wear off as the self-administration session ends. As a result of this 'sluggishness' electrochemical changes fall out of phase with responding for drug self-administration (see Di Ciano et al., 1995, 2001a). The relative 'sluggishness' of the changes in electrochemical signal compared with dialysate DA is also shown by a comparison of the results reported in the microdialysis study of Ranaldi et al. (1999) and the chronoamperometric study of Di Ciano et al. (2001a) both performed on rats self-administering unit doses of 0.25 mg/kg of amphetamine. Extinction of amphetamine self-administration resulted in an immediate fall of dialysate DA with first-order-like kinetics (Ranaldi et al., 1999). In contrast, the electrochemical signal continued to increase, although at a slower rate, for up to 90 min from the interruption of amphetamine availability (Di Ciano et al., 2001a). The increase in signal immediately preceding the reinforcer-associated decrease has been attributed by Gratton and Wise (1994) to conditioned anticipatory stimulation of DA transmission. However, the biphasic pattern observed in self-administering subjects, including the abrupt fall upon heroin injection, can be reproduced also after passive administration. This observation led Kiyatkin et al. (1993) to suggest that the anticipatory nature of the increase in signal is more apparent than real, being the result of fluctuations related to drug administration independently from its contingency upon a response. Consistent with this suggestion is the observation that if drug injection is prevented the signal continues to increase (Gratton and Wise, 1994). Kiyatkin and Stein (1993) have attributed the cyclic U-shaped pattern of change of the electrochemical signal in rats self-administering heroin to cyclic changes in blood pressure. This suggestion, however, has not been followed up by further studies.

More recently it has been consistently shown that the electrical stimulation of DA neurons results in alkaline pH shifts due to the increased extraction of CO_2 following the activity-induced stimulation of local blood flow (Venton et al., 2003). This change can be resolved from DA by voltammetric scanning but might contribute to the changes in DA-like chronoamperometric signals resulting from the activation of DA neurons.

7. DRUGS SURROGATES OF NATURAL REWARDS?

A widely held assumption in the fied of drug addiction is that addictive drugs mimic natural rewards and accordingly utilize the same neural pathways. This, however, is certainly an oversimplification. We have represented addictive drugs as surrogates of natural reinforcers but by this terminology we mean that drugs share only some aspects of natural reinforcers and not necessarily those that make them rewarding.

We have argued that psychostimulants, by releasing DA in the NAc, eventually mimic the appetitive/incentive component rather than the consummatory component of natural reward and the behavioral impact of conditioned stimuli rather than of unconditioned ones (Di Chiara, 1998). Consistent with this hypothesis is the circumstance that food reward induces hedonia independently from DA, in contrast with psychostimulant reward, which is mediated by the activation of DA transmission in the NAc shell. A problem with this hypothesis however derives from the observation that the ability to release DA in the

NAc shell is not a consistent property of conditioned stimuli. Thus, we have reported that olfactory as well as visual complex stimuli conditioned to food while releasing DA in the PFCX and in the NAc core, fail to release DA in the NAc shell. Similar observations have been made by Ito et al. (2000) with cocaine-conditioned stimuli. Other studies, however, including some recent ones from our laboratory, indicate that, under certain conditions, conditioned stimuli do release DA also in the NAc shell. Thus, we have recently shown that the same complex stimuli (plastic box filled with palatable food) that fail to release DA in the NAc shell when conditioned to food taste, do release DA in the same area when conditioned to subcutaneous morphine and nicotine (Bassareo et al., in preparation). In these experiments, drug-conditioning results in the acquisition of significantly stronger incentive properties than food-conditioning. Therefore, the failure of CS to stimulate NAc shell DA is not an absolute property of these stimuli but rather a function of the specific experimental conditions (nature of the reward, motivational state etc) under which conditioning takes place. In general, the ability to release DA in the NAc might be a function of the motivational impact of the CS and in this sense the property of stimulating DA transmission in the NAc shell would mimic the properties of particularly high-impact CS.

8. DOPAMINE AND DEPENDENCE THEORIES OF DRUG ADDICTION

Abstinence from chronic exposure to addictive drugs of different classes has been reported to induce a state of 'anhedonia' and dysphoria expressed by a reduction in the reinforcing properties of natural rewards and electrical brain stimulation (Markou and Koob, 1991). Associated to this state is a reduction of in vivo DA transmission in the NAc and in the activity of DA units in the ventral tegmentum that appears dissociated from the physical signs of abstinence (Pothos et al., 1991; Acquas and Di Chiara, 1992; Rossetti et al., 1992; Diana et al., 1993, 1995). Therefore, reduction of DA transmission in the NAc, like impairment of self-stimulation, seems to provide a more sensitive, longer lasting and more general sign of dependence than physical signs of abstinence. Reduction of DA transmission in the NAc following chronic drug exposure can be readily interpreted as the result of adaptive turning off of endogenous excitatory input on DA neurons secondary to the chronic drug-induced stimulation of DA transmission. However, it might also be the result of the unavoidable aversive state induced by abstinence. According to the first interpretation, reduction of DA release in the NAc would contribute to the negative state of abstinence while in the second case it would be a consequence of it. Consistent with the second possibility is the fact that the aversive state of abstinence generalizes to a pentylentetrazol stimulus (Emmett-Oglesby et al., 1990). Moreover, in the prefrontal cortex, abstinence is associated to an increase of DA release (Bassareo et al., 1995). It is notable that a pattern of increased DA release in the prefrontal cortex and a reduction in the NAc shell is also observed following exposure to aversive stimuli. These observations are consistent with the possibility that the changes in DA transmission in the NAc and in the prefrontal cortex associated with drug withdrawal are secondary to the inescapable aversive state of abstinence.

Early theories, by referring to opiate addiction as a model, placed major emphasis on physical dependence as a factor of drug addiction (Himmelsbach, 1943). More recent formulations, apart from providing a theory for the mechanism of tolerance and dependence (opponent process theory) (Solomon, 1977), have moved the emphasis from

physical dependence to motivational dependence and to withdrawal-induced anhedonia and dysphoria as motivational factors that maintain drug self-administration by a negative reinforcing mechanism (Koob et al., 1989, 1997). The advantage of this version of dependence theories of addiction is that motivational dependence, as assayed by electrical self-stimulation behavior in animals (Markou and Koob, 1991), has the properties of a factor common to different classes of drugs while physical dependence differs widely, as judged from the phenomenology of physical abstinence, from one drug class to the other.

Although it is difficult to negate that motivational dependence plays a role in drug addiction, it is unlikely to be necessary or sufficient. In fact, relapse of drug use takes place also after long periods of abstinence, when dependence is likely to have worn off; moreover, detoxification and recovery from a dependence state does not prevent relapse of drug abuse. Thus, drug-seeking takes place in spite of full detoxification and even under a full methadone maintenance regimen (Horns et al., 1975; Loimer and Schmid, 1992; de Vos et al., 1996). More recent formulations of the motivational dependence theory have attempted to correct this inadequacy by introducing the notion of a long-lasting change in the hedonic set-point (hedonic allostasis) resulting from nonassociative counteradaptive mechanisms (Koob and Le Moal, 2001). This hypothesis incorporates the notion of hedonia as a state dependent upon the tonic activity of DA neurons, a view not dissimilar from our view of a DA-dependent hedonia associated to a state of incentive arousal.

An advantage of the hedonic allostasis hypothesis is that it provides a basis for the strong comorbidity of drug addiction and depression. However, this relationship with depression is also the limit of the hypothesis. Thus, anhedonia induced by cocaine withdrawal has been proposed as a model of depression also on the basis of the observation that antidepressants reverse withdrawal-induced anhedonia; yet, antidepressants do not provide a treatment for drug addiction. Therefore it would appear that anhedonia is a condition associated to drug addiction but is not the factor that sustains its maintenance or its resumption after a long period of abstinence.

9. NONINCENTIVE ACCOUNTS OF DRUG ADDICTION

A review of the role of DA in drug addiction would not be complete without an account of theories of drug addiction that do not posit motivation at the center of the stage.

This is the case of the automatic responding view of Tiffany (1990) and of the aberrant habit-learning hypotheses (Everitt et al., 2001).

These two hypotheses are related, the second being a neuropsychobiological specification of the first. According to Tiffany (1990), drug addiction is a form of automatic responding independent from explicit/declarative knowledge of action-outcome relationships. Such implicit, rigid form of behavior would explain the compulsive character of drug seeking and taking under conditions of ad lib drug availability, which is often the case of legal substances such as tobacco or alcohol. This automatic form of responding ceases whenever some unpredicted event breaks the rigid relationship between the response and its outcome. This is the case with substance unavailability. Under these circumstances behavior is switched from the unconscious automatic modality into a conscious goal-oriented seeking of the substance. Craving is regarded as a cognitive expression of goal-oriented substance seeking behavior (Tiffany, 1990). Tiffany (1990) does not specify the nature of the automatic responding typical of drug addiction.

In principle it might be a 'Pavlovian habit' related to the establishement of a direct association between a Pavlovian CS and an UR (see Cardinal et al., 2004). Another possibility is that automatic responding is an instrumental habit related to learning of an instrumental stimulus-response (S-R) association. An instrumental habit is more likely to account for the relative flexibility of the behavior as indicated by the ability to rapidly switch to an explicit goal-oriented mode when the automatic responding is impaired.

This account of drug addiction might provide a phenomenological description of the behavior of the addict, but fails to identify its critical aspects. Thus, according to these accounts, the essential aspect of addiction is the automatic, habitual nature of responding.

This however is unlikely to be pathological per se. Transition into an automatic, habitual mode is typical of any scheduled responding and is the basis for the acquisiton of skills. However, it is unlikely that drug addiction could be envisioned as a skill in drug taking and seeking not only because the degree of skilfulness necessary for drug taking is elementary but also because learning of a skill does not necessarily involve a tendency to compulsively apply it or the emergence of craving when the possibility to perform it is impaired. Indeed, it is the occurrence of craving rather than the automatic or habitual nature of drug-taking that marks the difference between drug addiction and automatic or habit responding. According to Tiffany (1990), craving is unlikely to be a motivational factor of drug addiction because no relationship is found between craving and drug intake. This however is not unexpected if indeed craving is an indirect expression filtered as it is by cognitive processing of the motivation to take drugs. Relevant to this view are the brain imaging studies showing that craving is associated to activation of areas involved in the processing of motivational stimuli and states (Volkow et al., 2002). The view that regards craving rather than drug intake per se, as an essential feature of drug addiction is consistent with the DSM definition of drug addiction as related to the strength of the desire to take drugs.

10. DRUG ADDICTION AS ABNORMAL MOTIVATION

The definition of 'dependence' (i.e. addiction) provided by the DSM-IIIR (American Psychiatric Association, 1984) and DSM-IV (American Psychiatric Association, 1994) consists of a list of seven criteria or conditions, at least three of which should be present at the same time to allow a diagnosis of dependence. Two of these criteria correspond to physiological adaptive changes: (1) tolerance; (2) physical dependence; three of them correspond to loss of control over drug taking; (3) persistent desire and unsuccessful attempts to quit; (4) use of drugs in larger amounts and for longer periods than intended; (5) continued use in the face of medical, familial or social problems; finally, two criteria correspond to focussing of instrumental behavior over drug taking; (6) important social, familial and recreational activities given up or reduced because of drug-seeking; (7) expenditure of a great deal of time and activity in relation to drugs. This definition involves conditions (items 3–7) that can be indexed as an expression of abnormal drug motivation, i.e. of the strong control that the drug acquires over the subject's behavior and of the restriction of the subject's range of activities to drug-seeking and drug-taking. It is notable that, although tolerance and physical dependence are among the seven items that the DSM-IIIR and DSM-IV indicate as useful for a diagnosis of dependence, their presence is not necessary; thus, one can diagnose dependence by the presence of three of the five items that are related to abnormal drug motivation. On the other hand, according

to DSM-IIIR and DSM-IV, tolerance and physical dependence are not sufficient to diagnose dependence since they account for only two among the three items necessary for diagnosis, the third being, again, an item of abnormal drug motivation.

This analysis shows that DSM-IIIR and DSM-IV attribute to the loss of control over drug taking the highest rank compared with tolerance and physical dependence, being both necessary and sufficient for dependence. The importance attributed to excessive or abnormal motivation to take drugs in the operational definition of dependence and addiction is directly relevant to the issue of the biological bases of these conditions and is consistent with the fact that the dependence liability of drugs is associated with the property of serving as positive reinforcers, that is, of promoting the emission of behaviors that are followed by the occurrence of the Drug (Johanson, 1978). Indeed, from a formal point of view, drug dependence and addiction can be reduced to a case of drug reinforcement; this, however, does not eliminate but actually increases the need for devising some operational criteria for distinguishing addiction from normal reinforcement in animals. Consistent with the above definition, drug addiction can be conceptualized as a disorder of motivation characterized by the excessive control over behavior exerted by drugs through the acquisition of Pavlovian conditioned stimuli acting as incentives of drug-taking behavior (Wikler, 1973; Goldberg, 1976; Stewart et al., 1984; Childress et al., 1988; O'Brien et al., 1992; Robinson and Berridge, 1993; Di Chiara, 1998).

10.1. DOPAMINE AND THE EXPRESSION OF DRUG ADDICTION

Various theories of drug addiction have capitalized on the assumption that DA mediates the incentive effects of drug-conditioned either discrete or contextual stimuli. According to Stewart et al. (1984), release of DA by drug-conditioned stimuli provides the incentive for responding for drug self-administration. According to the related theory of Robinson and Berridge (1993) DA, released in the mesocorticolimbic system by drug-conditioned stimuli, mediates an 'incentive salience attribution' process in series between the stimulus and the response.

10.1.1. Sensitization of drug-induced activation of DA transmission: the incentive-sensitization theory

Repeated drug exposure is known to induce sensitization to the behavioral stimulant effects of the drug along with sensitization of drug-induced presynaptic stimulation of DA transmission in the ventral and, to a lesser extent, in the dorsal striatum (Kalivas and Stewart, 1991; Robinson and Berridge, 1993) as well as adaptive changes in gene expression and synaptic plasticity (Hyman and Malenka, 2001; Nestler, 2001). Robinson and Berridge (1993), mainly on the basis of studies with psychostimulants, have proposed an incentive-sensitization theory of drug addiction. This theory posits that repeated drug exposure induces a state of sensitization of mesocorticolimbic DA neurons; as a result of this adaptive, nonassociative change drug-related stimuli become more effective in stimulating DA transmission in mesocorticolimbic areas and in triggering craving, regarded as an abnormal incentive state (abnormal wanting) (Robinson and Berridge, 1993).

A basic difference between the incentive-sensitization and the incentive learning hypothesis is that, while the first views addiction as a disorder of the expression of the incentive properties of stimuli, the second envisions it as a disturbance of the acquisition of those properties.

Moreover, while in the incentive learning hypothesis abnormal motivation is the result of the abnormal properties of the stimulation of DA transmission by the drug, in the incentive sensitization theory abnormal motivation is the result of the excessive activation of DA transmission by stimuli conditioned to the drug. An excessive activation of DA transmission by drug-conditioned stimuli is also predicted by the incentive learning hypothesis but as secondary to the excessive impact acquired by drug-conditioned stimuli as a result of abnormal incentive learning.

Various studies support the notion that behavioral sensitization is associated to an increase in the reinforcing properties of drugs of abuse (Vezina, 2004) as indicated by increase in the break point in progressive ratio schedules (Mendrek et al., 1998, Vezina et al., 2002) and in the rate of acquisition of drug self-administration (Piazza et al., 1989, 1990; Horger et al., 1990, 1992; Valadez and Schenk, 1994; Pierre and Vezina, 1998). Sensitization also facilitates the acquisition of drug-conditioned place preference (Lett and Grant, 1989, Shippenberg and Heidbreder, 1995; Shippenberg et al., 1996).

In agreement with the nonassociative nature of incentive-sensitization, amphetamine sensitization potentiates DA release in the amygdala in response to a stimulus predictive of sucrose pellets (Harmer and Phillips, 1999) and enhances appetitve conditioning of a stimulus paired to sucrose reward (Harmer and Phillips, 1998). Notably, sensitization did not enhance the secondary reinforcing properties of the stimulus. Amphetamine sensitization also facilitates sexual behavior and potentiates DA release during copulation (Fiorino and Phillips, 1999). Cocaine sensitization increases the stimulation of responding with conditioned reinforcement elicited by intra-accumbens amphetamine (Taylor and Horger, 1999). Finally, it has been recently reported that cocaine sensitization increases the basal activity of A10 DA neurons (Marinelli and White, 2000).

These observations are consistent with the prediction that sensitization induced by repeated drug exposure increases the basic responsiveness of DA neurons to stimuli (Robinson and Berridge, 1993). However, this conclusion, while consistent with the prediction of the theory, turns out to be deleterious for its validity as a model of human addiction. In fact, since sensitization increases the incentive properties of any appetitive stimulus, not only of drug-related ones, it can hardly account for a cardinal feature of drug addiction, namely that the excessive impact over behavior exerted by drug-conditioned stimuli is reciprocated by a reduced impact by stimuli conditioned to nondrug rewards.

In order to circumvent this difficulty Robinson and Berridge (1993) have introduced the concept that the expression of sensitization is under conditioned stimulus control, as in the case of context-dependent sensitization. In this condition sensitization is expressed as an increased behavioral stimulant effect of the drug. Craving, however, is classically elicited by exposure to drug-conditioned stimuli rather than to the drug itself (Stewart et al., 1984). Moreover, craving is strictly drug related i.e. is elicited by stimuli conditioned to the drug and not by conditioned stimuli in general. This however does not appear to be the case with sensitization. Thus, repeated exposure of rats to amphetamine results in sensitization of the facilitation by a Pavlovian CS of instrumental responding for sucrose by intraNAc shell infusion of amphetamine (Wyvell and Berridge, 2000, 2001). According to this model, the behavior of a cocaine addict with cocaine on board should be compulsively instigated by any incentive stimulus occurring in the environment no matter if conditioned to the drug or to any other reward. This is clearly not the case in the real world of drug addicts, whose behavior is highly focussed on drug cues to the exclusion of nondrug cues (Tiffany, 1990; O'Brien et al., 1992).

10.1.2. Does behavioral sensitization takes place in human addiction?

From a more practical point of view, the major problem with the incentive-sensitization theory of drug addiction is the lack of evidence for its occurrence in the human addict. Thus, Volkow et al. (1997b) showed that in cocaine postaddicts no behavioral (as expressed by drug-induced high) or biochemical sensitization (as expressed by drug-induced increase of extracellular DA in the striatum) can be demonstrated. As a matter of fact, a decrease in these measures was observed. These observations put a major question mark on the validity of the DA sensitization theory as an explanatory framework of drug addiction.

One might argue that the studies by Volkow et al. (2002) referred to the whole striatum and not specifically to its ventromedial subdivision, corresponding to the nucleus accumbens of the rat, where most animal studies have been performed. However, in the rat a sensitization of striatal DA responsiveness to drug challenge has always been found whenever it has been looked for. A recent study in the monkey reported sensitization of cocaine-induced increase of DA not only in the ventromedial striatum but also in the striatum as a whole, when data from all striatal microdialysis probe placements, including central and dorsolateral placements, were pooled together (Bradberry, 2000). No significant sensitization was obtained if only samples from the dorsal striatum were specifically considered. In this study Bradberry (2000) noted that in the monkey, sensitization was obtained after low unit doses of cocaine self-administered twice daily with an interval of 100 min. Also in rats, biochemical sensitization typically takes place after mild schedules of exposure to psychostimulants. This circumstance raises the question of whether a more aggressive exposure to cocaine, like that typical of cocaine addicts, would also result in biochemical sensitization. The fact that in cocaine postaddicts no sensitization of drug-induced DA responsiveness was observed (Volkow et al., 1997b) would suggest that no sensitization will be observed in an animal model of cocaine intake mimicking that of human addiction. Evidence that this might indeed be the case is recently provided by studies of Bradberry et al. (2003) showing that, in contrast to milder ones, more aggressive schedules of cocaine exposure fail to induce sensitization of cocaine-induced increase of DA in the striatum in the monkey.

These observations raise the question of which patterns of cocaine intake, among those utilized by humans, are modeled by the schedules of cocaine exposure currently utilized to induce sensitization in animal studies. Particularly relevant to this issue is the recent study by Koob et al. showing that while a mild schedule of cocaine self-administration results in behavioral and biochemical sensitization to cocaine, binge-exposure to cocaine does not. Given these premises, biochemical sensitization to cocaine might be more easily demonstrated in subjects self-administering the drug irregularly as hydrochloride and by the nasal route, rather than as a base and in a binge-like fashion via the inhalatory route. One might even ask to what extent, given the mild pattern of exposure by which cocaine is capable of inducing sensitization, this change should be taken as a neurobiological expression of cocaine addiction. That this might be indeed the case is suggested by the fact that sensitization of amphetamine-induced central effects has been reported in normal volunteers, naive to the drug and exposed two or three times, 48 h apart, to single, low doses (0.25 mg/kg) of amphetamine by the oral route (Sax and Strakowski, 2001). Although these observations are difficult to compare with those of Volkow et al. (1997b), due to differences in the drug studied, route of administration and behavioral parameters recorded, they are consistent with the present suggestion, i.e. sensitization is obtained with

mild schedules of drug exposure that do not mimic the patterns of self-administration typical of drug addiction.

One might ask if the parameters under consideration in the studies by Volkow et al. (2002) i.e. drug-induced increase of extracellular DA in the striatum and drug-induced high, are indeed the right biochemical and behavioral parameters, respectively, to consider when testing the role of sensitization in drug addiction. For example one might argue that activation of DA transmission by drug-related stimuli rather than by the drug itself, and craving rather than drug-induced high are the appropriate measures to take into consideration for testing the sensitization hypothesis. Indeed, according to the incentive-sensitization theory, craving is the expression of sensitized release of DA in the mesocorticolimbic system by drug-conditioned stimuli (Robinson and Berridge, 1993). However, an alternative possibility is that craving is the result of abnormal incentive learning of drug-conditioned stimuli. Therefore, testing of the incentive-sensitization hypothesis requires the use of measures as independent as possible from associative learning mechanisms. Stimulus-conditioned craving and conditioned release of DA do not fulfill this requirement. A further caveat against the use of craving as an index of sensitization is that craving is not present in nonaddicted subjects and therefore cannot be taken as a measure of sensitization homologous to hypermotility in animals. Indeed, as the DA dependency of craving is the issue under test, craving cannot be utilized as an a priori behavioral correlate of drug-induced stimulation of DA transmission. A general argument, often utilized by the proponents of the sensitization hypothesis, is that hedonia is not expected to be increased by sensitization, since it is essentially independent of DA. As euphoria, according to this position, is an expression of hedonia (liking), self reported measures of euphoria are not expected to increase in sensitized subjects. This assumption however, is challenged by the observation by Volkow et al. (2002) and by Drevets et al. (2001) that the self-reported high/euphoria in response to a psychostimulant is a direct function of the change in DA transmission in the striatum, and in particular in the ventral striatum. Therefore, not only are self-reported high/euphoria measures appropriate as an expression of the activity of DA transmission in the striatum but, contrary to the prediction of the sensitization theory of an increased expression of DA-dependent measures, these measures are not sensitized in cocaine postaddicts. It is worth noting that euphoria is not the only DA-dependent effect that does not undergo sensitization in cocaine addicts for this is also the case for restlessness, a more motor expression of DA activity (see Volkow et al., 1997b, Table 1).

From the above discussion, we conclude that incentive-sensitization is not a model of drug addiction and one might wonder of which condition this neuroplasticity change might be a model. A discussion of this issue, however, is beyond the scope of this review.

10.2. DOPAMINE AND THE ACQUISITION OF DRUG ADDICTION: THE PAVLOVIAN INCENTIVE LEARNING HYPOTHESIS

Consistent with the role of NAc DA in motivation and with the property of addictive drugs to stimulate DA transmission in the Nac, is the idea that drug addiction is a condition of disturbed motivation related to drug-induced stimulation of NAc DA.

Among existing theories the one that explicitly links the disturbance of motivation to the property of addictive drugs to stimulate DA transmission in the NAc, and in particular in the NAc shell, is the abnormal Pavlovian incentive learning hypothesis of drug

addiction (Di Chiara, 1998, 1999). This hypothesis explains the motivational abnormality of drug addiction as the result of the excessive strenghtening of Pavlovian stimulus-drug associations by repeated drug-induced stimulation of DA transmission in the NAc. This excessive strengthening, in the case of addictive drugs, would arise from the circumstance that drug-induced stimulation of DA transmission in the NAc shell lacks the adaptive properties of the stimulation induced by nondrug (food) reward; these properties are the single-trial habituation and the inhibition by exposure to conditioned stimuli (Bassareo and Di Chiara, 1997, 1999a,b; Bassareo et al., 2002). Thus, a second exposure to the same taste, either 4 or 24 h after the first, fails to release DA in the NAc shell while still releasing DA in the medial prefrontal cortex. Moreover, preexposure to an olfactory stimulus conditioned to the taste of food prevents taste-induced release of DA in the NAc shell but not in the medial prefrontal cortex (Bassareo and Di Chiara, 1997, 1999a,b; Bassareo et al., 2002). No such negative adaptive mechanisms take place in the case of drug-induced stimulation of DA transmission in the NAc shell. Thus, a 10% ethanol solution in water infused into the mouth elicits two peaks of DA release, an early one related to its taste and associated to mixed edonic/aversive behavioral reactions, and a late one, coincident with peak ethanol levels in dialysates. On a second exposure, 24 h later, the early DA rise underwent complete habituation in spite of persistence of hedonic reactions and actual loss of aversive reactions. The late DA rise, instead, remained, being actually potentiated (Bassareo et al., 2003). Thus, habituation differentially affected the release of DA induced by central ethanol action and by ethanol taste.

Another way by which drugs can abnormally affect behavior is by their influence on the acquisition of conditioned properties by associated stimuli.

We have now completed a series of experiments comparing the response of DA transmission in the NAc shell, core and PFCX to food and morphine-conditioned stimuli (Bassareo, DeLuca and Di Chiara, in preparation).

Rats underwent three trials consisting of a 10 min exposure to a perforated cylindrical box made of sky-blue transparent plastic (height 8 cm; diameter 6 cm), filled up with 8 g of a highly palatable snack food (Fonzies Filled Box, FFB) followed (conditioned) or randomly preceded (pseudoconditioned) by administration of morphine (1.0 mg/kg sc) or saline. In the case of food only two groups, a conditioned (Fonzies presentation for 20 min, 10 min after FFB) and an unconditioned group (regular rat chow presentation) were studied. The day following the last day of training rats were implanted with microdialysis probes in the NAc shell and core. The day after the implant of the probes dialysate DA was monitored during a 40 min exposure to the FFB followed by challenge with morphine (0.5–1.0 mg/kg sc). Incentive reactions to the FFB (orienting reactions, approach responses and consummatory attempts focussed on the FFB) were scored during a 40 min presentation of the FFB (Bassareo and Di Chiara, 1997).

Exposure to the FFB elicited incentive reactions both in in morphine-conditioned and in food-conditioned rats. Incentive reactions to the FFB were milder and shorter lasting in the pseudoconditioned and unconditioned groups as compared to conditioned ones. In unconditioned rats no change in dialysate DA was observed in the NAc shell or core upon presentation of the FFB while Fonzies feeding elicited the expected increase of DA in the shell and in the core (Fig. 6). In unconditioned and pseudoconditioned rats morphine increased DA in the NAc shell (Fig. 7) but not in the core (Fig. 8). In food-conditioned rats neither the CS nor Fonzies feeding affected dialysate DA in the NAc shell (Fig. 8), consistently with previous reports (Bassareo and Di Chiara, 1997). In morphine-conditioned rats both the CS and morphine (1 mg/kg sc) significantly increased dialysate

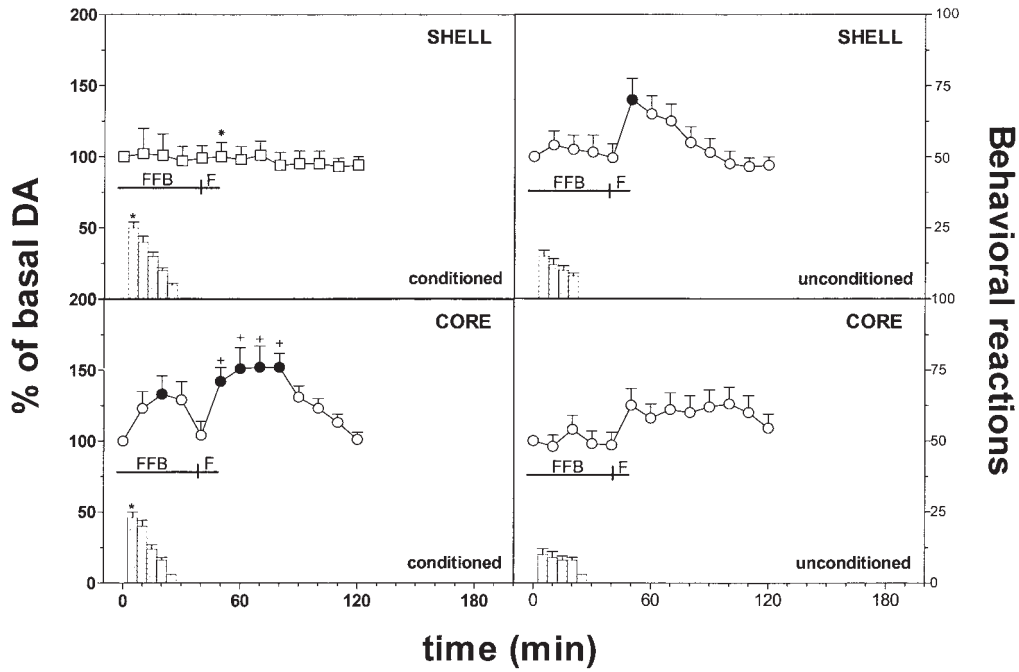

Fig. 6. Effect of a complex stimulus (Fonzies Filled Box, FFB) and Fonzies feeding on behavior and DA output in dialysates from NAc shell and core in Fonzies-conditioned and unconditioned rats. Results are mean ± SEM of the results obtained in at least four rats. Filled symbols: $p < 0.05$ with respect to basal values; *: $p < 0.05$ with respect to unconditioned rats; +: $p < 0.05$ with respect to conditioned rats implanted in the NAc shell.

DA in the NAc shell (Fig. 7). The effect of morphine in the NAc shell following preexposure to the CS was actually potentiated (Fig. 8). Vice versa, in the NAc core DA increased in response to the Fonzies-conditioned CS (Fig. 6) but not to the morphine-conditioned CS (Fig. 7). As expected, DA increased in response to Fonzies feeding in the NAc core of the conditioned group.

These studies show that presentation of a conditioned stimulus, while inhibiting the stimulatory response of of NAc shell DA to food reward, actually potentiates the response to drug reward. Moreover, while drug-conditioned stimuli elicit a sustained release of DA in the NAc shell but not in the NAc core, food-conditioned stimuli release DA in the NAc core but not in the shell.

These observations demonstrate the striking differences existing between drug-conditioned and food-conditioned stimuli in their differential ability to affect DA transmission in the NAc shell and core and the different adaptive consequences exerted by these conditioned stimuli on the ability of drug and food reward to stimulate DA transmission in the NAc shell.

These differences seem directed as a whole toward a higher stimulatory impact of drug reward and of drug-conditioned stimuli on NAc shell as compared to NAc core DA transmission. From this point of view the differences in the consequences of associative (conditioning) and nonassociative (sensitization) manipulations on the responsiveness of DA transmission to addictive drugs is notable. Thus, in contrast with conditioning, sensitization increases the stimulatory impact of drugs on NAc core DA while reducing

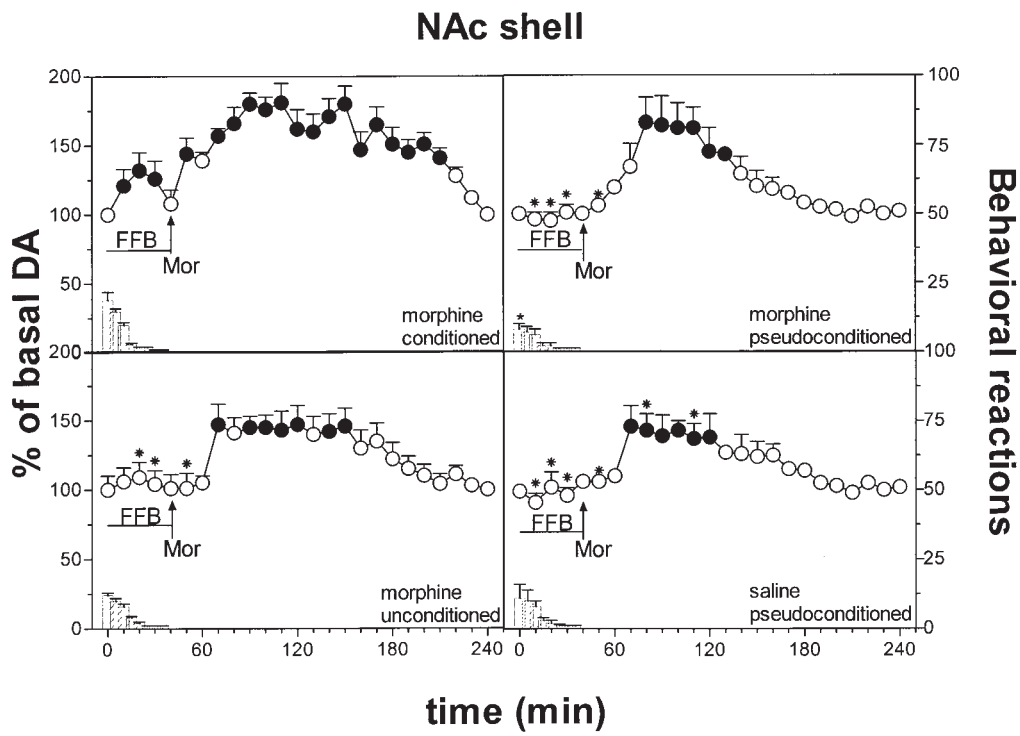

Fig. 7. Effect of a complex stimulus (Fonzies Filled Box, FFB) and morphine (1 mg/Kg s.c.) on behavior and DA output in dialysates from NAc shell in unconditioned, morphine-conditioned, morphine-pseudoconditioned and saline-pseudoconditioned rats. Results are mean ± SEM of the results obtained in at least four rats. Filled symbols: $p < 0.05$ with respect to basal values; *: $p < 0.05$ with respect to conditioned rats.

that on the NAc shell (Cadoni and Di Chiara 1999; Cadoni and Di Chiara 2000; Cadoni et al., 2000; Cadoni et al., 2003). The behavioral sequelae of the actions of drug-conditioned stimuli on DA transmission can be deduced from the functions attributed to NAc shell DA on the basis of previous studies. These are mainly related to strengthening of Pavlovian incentive learning and of consolidation of Pavlovian CS-UCR associations as well as potentiation of the incentive arousing properties of drug-conditioned stimuli on instrumental responding for drug (Di Chiara, 2002).

We hypothesize that the excessive activation of these processes as a result of dysadaptive stimulation of DA transmision in the NAc shell by drugs of abuse and by their associated stimuli can account for the pattern of compulsively focussed motivation on drugs and drug-related stimuli typical of drug addiction.

11. A GENERAL THEORY OF ABNORMAL MOTIVATION AS DISADAPTIVE RESPONSIVENESS OF NAC SHELL DA

Drug addiction might be just a special case of abnormal motivation secondary to nonadaptive responsiveness of NAc shell DA to primary appetitive stimuli. Thus, other disturbances of motivated behavior characterized by compulsion and excessive reactivity

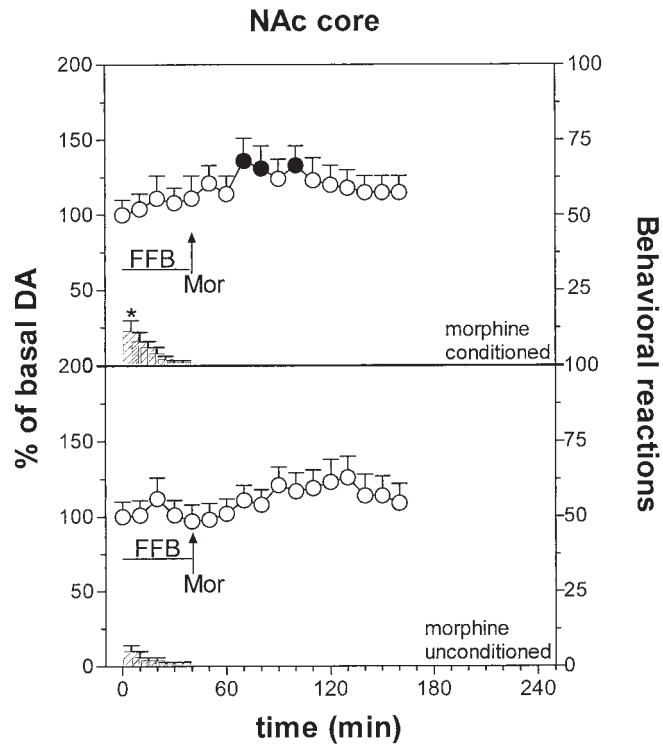

Fig. 8. Effect of a complex stimulus (Fonzies Filled Box, FFB) and morphine (1 mg/Kg, s.c.) on behavior and DA output in dialysates from the NAc core of unconditioned and morphine-conditioned rats. Results are mean ± SEM of the results obtained in at least four rats. Filled symbols: $p < 0.05$ with respect to basal values.

to stimuli conditioned to nondrug rewards (food, sex, gambling), could be accounted for by a defect in the responsiveness of NAc shell DA to the reward. Specifically, one could hypothesize that in affected individuals NAc shell DA responsiveness fails to habituate to the repeated exposure to specific rewards with resulting abnormal Pavlovian incentive learning and acquisition of excessive incentive properties by stimuli conditioned to that reward. Experimental testing of this hypothesis depends upon the availability of adequate animal models. In the case of feeding disorders, one might capitalize on the circumstance that food deprivation abolishes the adaptation of DA responsiveness in the NAc shell to palatable food (Bassareo and Di Chiara, 1997, 1999). In this case, however, the picture is complicated by the fact that food deprivation also increases the impact of food as a reward. Recently we have found that morphine sensitization results in abolition of habituation of DA responsiveness in the NAc shell to palatable food (Bassareo et al., in preparation). This observation, while consistent with the existence of commonalities between drug and nondrug rewards, might provide a model for experimental testing of the above hypothesis.

12. CONCLUSION

DA plays a fundamental role in behavior motivated by rewards and reward-related stimuli. Release of DA in the NAc shell by Pavlovian stimuli induces an appetitive state of incentive arousal (state-hedonia, euphoria) that facilitates the rate of current instrumental

behavior, the acquisition and expression of secondary reinforcement and the reinstatement of previously extinguished instrumental responding as well as the consolidation of mnemonic traces of salient stimuli to be associated with affective states.

This incentive arousal state is induced by the occurrence of novel, unpredicted primary rewards and is subjected to negative adaptation (habituation). All drugs of abuse induce, to a different extent depending on the pharmacological class they belong to, such incentive arousal state as a result of their ability to increase extracellular DA in the NAc shell. This property, however, in contrast to nondrug rewards, is not subject to adaptive regulation (habituation).

The property of drugs that allows these adaptive differences with conventional reinforcers is a basic one: drugs enter the brain and directly activate or disinhibit DA neurons. In contrast, for their effects on DA neurons, conventional reinforcer drugs depend on the stimulation of a long chain of neurons triggered by stimulation of peripheral sensory receptors.

Such dysadaptive stimulation of DA transmission in the NAc shell by repeated, response-contingent exposure to drugs of abuse would result in the motivational abnormalities typical of addiction, namely compulsive focussing on drugs and drug-related stimuli at the expenses of more conventional nondrug rewards.

13. ABBREVIATIONS

CS	conditioned stimulus
CTA	conditioned taste aversion
CPP	conditioned place preference
DA	dopamine
DAT	dopamine transporter
EC	extracellular compartment
ICSS	intracranial self-stimulation
Nac	nucleus accumbens
PFCX	prefrontal cortex
PIT	transfer from Pavlovian to instrumental
OT	olfactory tubercle
6-OHDA	6-hydroxydopamine

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CHAPTER VII

Role of cortical and striatal dopamine in cognitive function

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ABSTRACT

The role of the central dopamine systems in cognitive function is reviewed. The contribution of the mesolimbic, mesostriatal and mesocortical dopamine systems is considered in functions such as learning, working memory and attention, using electrophysiological and neuropharmacological, as well as psychopharmacological evidence. This experimental evidence in animals is further assessed in the context of a review of how dopamine modulates cognition in humans, as inferred from psychopharmacological and functional imaging studies, for normal subjects and for patients with disorders such as Parkinson's disease, schizophrenia and ADHD. Finally, new possible approaches for developing this field in the future are identified, notably those based on computational modeling and functional genomics.

1. INTRODUCTION

The mapping of the central dopamine (DA) pathways into anatomically discrete mesostriatal, mesolimbic and mesocortical systems, the cloning of their main receptors and the identification of their cell signaling pathways, have all raised relevant questions about the functions of this important neuromodulatory neurotransmitter. The implication of DA in Parkinson's disease has suggested for this neurotransmitter important functions within the motor system to regulate the output of the striatum. But equally, identifying mesolimbic DA as a major element in the reinforcing effects of many drugs of abuse, especially the psychomotor stimulants amphetamine and cocaine, indicates apparently different functions within a parallel circuitry. Based on this involvement in drug reinforcement, some authors (e.g. Wise, 1982) have earlier implied that DA release is associated with subjective hedonistic responses and the effective use of DA receptor antagonists in the treatment of psychotic symptoms such as delusions and hallucinations, is certainly apparently consonant with the view that DA activity can affect subjective elements of presumably aberrant cognitive processes. However, burgeoning evidence of a role for DA in aspects of frontal lobe function such as working memory, suggests an even closer and more direct relationship to cognition. While it is tempting to ascribe these three main functional correlates; motor function, reinforcement and higher order cognition to independent modulation by DA of parallel circuitry in the mesostriatal, mesolimbic and

mesocortical systems respectively, this convenient tripartite categorization may not be the best way to analyze the role of DA. This is because these apparently distinct behavioral functions themselves are inter-related; for example, a role for DA in reinforcement may also be associated with important functions in learning, memory and decision-making cognition. There are also intimate anatomical relationships between the cortex and the striatum, in the form of cortico-striatal 'loops' which have a degree of segregation as well as serial interconnection (Alexander et al., 1986; Haber et al., 2000) which means that their associated DA systems must sometimes be modulating similar streams of information processing. Moreover, it is probably a gross simplification not to assume, for example, that the dorsal striatum has cognitive, as well as motor functions. In this chapter, we attempt to identify a crucial role of DA in cognition that is consistent with these complexities. This will entail a theoretical perspective that includes, within the wide category of cognitive functioning, the processes of attention, learning and different aspects of memory. A key issue is under what state or conditions are the central DA system active and how does this activity affect cognition. As there are considerable neurochemical data indicating that central DA is affected by such factors as stress, this question may be equivalent to understanding the relationship between such states as stress or mood and cognition. Whether an integrated perspective of how DA affects these interwoven behavioral functions is possible may also depend on a closer understanding of how the DA activity affects the functioning of its terminal domains at a cellular level. We will also be considering the recent attempts to provide integrative computational models of the actions of DA that utilize, to varying extents, data on its cellular actions in different functional contexts, including reinforcement learning and working memory.

2. A ROLE FOR DA IN LEARNING AND MEMORY

2.1. ELECTROPHYSIOLOGICAL EVIDENCE

The implication of DA in reinforcement mechanisms within the nucleus accumbens suggests that stimulus-reward learning may occur there, as also postulated by White (1989). Accordingly, there has been increasing interest in the possible contribution of DA to reinforcement learning that has been given impetus by a combination of electrophysiological findings and computational modeling approaches (Houk et al., 1995; Montague et al., 1996; Schultz et al., 1997; Schultz and Dickinson, 2000). The critical issue is not that DA contributes to reinforcement per se, but to specify its exact role in associative learning. Precise data concerning the possible coding of reinforcement by DA neurons have been obtained from experiments in which their activities is recorded in alert monkeys while they perform in situations where their behavior earns food rewards (Schultz, 1992). In such experiments, the DA neurons in the midbrain ventral tegmental region respond with short, phasic activity when monkeys are presented with appetitive stimuli. The DA neurons are also transiently activated by novel stimuli that elicit behavioral orienting over their first few presentations. However, some aversive stimuli, such as air-puffs to the hand, or drops of saline to the tongue, are not very effective in eliciting firing (although there are some indications that such firing may also be anatomically separable from the units that reliably respond to rewards, see Levita et al., 2002). When repeated presentations of food reward are reliably predicted by other cues such as lights or noises, the activity of the DA neurons is advanced temporally to the time

of onset of these conditioned stimuli (CS) and responding to the reward stimulus itself is no longer present. However, if reward is omitted, the activity of the DA neuron is depressed at exactly that point in time at which it would normally have occurred, suggesting that it contributes to an internal representation of the reward. There is no evidence that DA-ergic activity represents sensory properties of the reinforcer (e.g. its precise visual or olfactory nature), which are presumably encoded by other neural networks in nonstriatal structures (for example, the orbitofrontal cortex).

These findings have been interpreted as being consistent with theories of associative conditioning such as that of Rescorla and Wagner (1972), which lay emphasis on the importance of the predictability of the unconditioned reinforcer (see Schultz and Dickinson, 2000). Learning occurs as a consequence of reducing error feedback signals, such that when reward is completely predictable, no further learning occurs. The activity of the DA neurons appears to provide a 'teaching signal', supplying information about the expected time and magnitude of reinforcement (Montague et al., 1996; Schultz et al., 1997). These teaching signals have the capacity to affect behavior by altering the synaptic weights of neural networks within terminal structures such as the striatum. Delays of reinforcement could be mediated by biochemical changes initiated in striatal neurons by the binding of DA to its receptors (Houk et al., 1995). Recent evidence has provided considerable support for the idea that delay of reinforcement is a vital parameter in the DA-dependent functions of the nucleus accumbens. For example, excitotoxic lesions of the core subregion of the nucleus accumbens induce a marked preference for immediate, small reinforcers (1 food pellet) versus delayed large reinforcers (4 pellets), although rats with such lesions are capable of distinguishing small versus large reinforcers per se (Cardinal et al., 2001). Moreover, there is also evidence that this preference is modulated under certain conditions by d-amphetamine and dopamine receptor antagonists (Cardinal et al., 2000).

This circumstantial electrophysiological evidence for a role for central DA-ergic mechanisms in learning must be considered in the light of other data indicating more immediate effects of DA-ergic activity that directly affect processing in its terminal regions, producing, for example, general changes in locomotor activity (e.g. in the rat, Kelly et al., 1975). A second issue is the extent to which the electrophysiological and neurocomputational findings are supported by direct evidence that DA plays a causal role in learning and memory processes. It is possible that the changes in activity in DA neurons reflect plastic changes occurring elsewhere, being consequences rather than causes of learning. The changes in DA activity would then still play a crucial role in behavior, but may not be necessary for learning. For example, Redgrave et al. (1999) have suggested that the changes in DA activity might provide a signal to switch from one form of behavior to another (e.g. from lever pressing to food consumption), consistent with established roles for striatal DA in the control of behavioral orienting and attention.

At the neurobiological level of analysis, there is also a lack of consensus that DA-ergic activity in the ventral striatum is necessary for, or augments, processes of neuronal plasticity, as exemplified for example, by long term potentiation (LTP) (Pennartz et al., 1994, 1995), although positive data exist for a DA-ergic modulation of LTP via D1/D5 receptors for the hippocampus CA1 area in vitro (Otmakhova and Lisman, 1996), hippocampal-prefrontal cortex synapses in vitro (Otani et al., 1998; Blond et al., 2001) and in vivo (Gurden et al., 1999, 2000). While other, possibly DA-dependent, forms of neuronal plasticity have been demonstrated within the dorsal striatum (including long

term depression (Calabresi et al., 1995) their possible relevance to behavioral learning is also not well established (but see Graybiel, 1995).

Whether the single unit and LTP evidence in fact have behavioral correlates in learning could be resolved by evidence that pharmacological interventions which reduce or enhance DA activity should produce predictable changes in learning- impairment or facilitation, respectively. In fact, as will be seen, the apparently important roles that DA has in behavioral performance means that it is more difficult to provide this decisive evidence of its role in learning than might at first be thought. Converging lines of evidence are required to resolve these issues, including tests which isolate causal relationships between DA and behavior.

2.2. NEUROPHARMACOLOGICAL EVIDENCE: NEUROCHEMICAL MONITORING

One of the two major neuropharmacological approaches for investigating the functions of DA monitors fluctuations in extracellular DA that occur in behavioral situations using in vivo dialysis or voltammetry. The DA levels change as a consequence of altered release and re-uptake mechanisms and reflect not only synaptic concentrations, but also gradients of local concentrations distal from the synapses themselves. While potentially providing important converging evidence for the role of DA neurons in associative processes, these monitoring techniques do so over a much longer time scale (minutes in the case of in vivo dialysis) than in the case of electrophysiological recording from identified DA neurons. Microdialysis offers the considerable advantage over voltammetry (and single unit neurophysiology) of chemical specificity, but the disadvantage of poor temporal resolution. This means that the capacity to establish temporal precedence of the effect of one event over another is diminished, and thus compromises the use of the technique for establishing causal relationships between behavioral contingencies and chemical events. Moreover, this lack of temporal resolution also means that any change may reflect 'rebound' or compensatory processes that overwhelm the immediate effect of the discrete event.

DA neurons appear to be responsive to a variety of stimuli and states, as well as pharmacological challenges. Consistent with Schultz' electrophysiological data in monkeys, presentation of food or water to rats can lead to increases in extracellular DA, sometimes in the dorsal, as well as the ventral striatum (see review by Blackburn et al., 1992). Moreover, the responses are greater if food is presented in an intermittent, periodic manner than all at once (Salamone et al., 1994). Also consistent with Schultz' evidence is that DA neurons show changes in activity to previously neutral environmental stimuli (e.g. lights and auditory tones) which are conditioned to important events such as food delivery, although there are many apparent discrepancies in this area, perhaps related to such factors as degree of food deprivation. Less obviously consistent with the electrophysiological data, foot shock can also increase extracellular striatal DA whereas some stimuli (e.g. loud noises leading to startle responses (Humby et al., 1996) and aversively conditioned taste stimuli (Mark et al., 1991) produce reductions in ventral striatal DA.

One elegant study (Bassareo and Di Chiara, 1999) has shown that the medial, so-called 'shell' region of the nucleus accumbens responds to presentations of novel palatable food with increased concentrations of extracellular DA, a response which habituates even though the rat may be consuming more food with repeated presentation. The DA response

may therefore be related to the salience of the food, and possibly, at a behavioral level, to the motivational excitement likely to occur in the presence of a highly appetitive reinforcer. DA levels in the medial prefrontal cortex increase in parallel, but fail to show such clear-cut habituation (Bassareo and Di Chiara, 1997). Conditioned stimuli (largely olfactory) predicting food presentation, also elevate DA in the medial prefrontal cortex, a response not initially seen in the nucleus accumbens (Bassareo and Di Chiara, 1997). However, a later study (Bassareo and Di Chiara, 1999) clarified the situation by showing how the conditioned stimuli led to increases in DA concentrations in the core region of the nucleus accumbens, but inhibited the response to food itself in the shell. These experiments suggest that the mesolimbicocortical DA system is modulating different aspects of appetitive behavior; possibly aspects of the representation of the unconditioned reinforcer in the shell, and those of the conditioned stimulus or reinforcer in the core regions of the nucleus accumbens. The latter results are broadly consistent with the electrophysiological data of Schultz, in showing some connection between associative mechanisms and striatal DA transmission, even though the methods employed are probably monitoring different temporal modes of DA-ergic transmission, in terms of tonic (steady-state) extracellular levels and phasic release, associated with burst firing patterns (Moore et al., 1999).

However, it is nevertheless problematic to resolve the question of whether such changes are causally involved in the associative process itself, since they could reflect the expression of some behavioral correlate of learning (such as 'motivational excitement'). An alternative way of addressing this issue is to utilize preparations of Pavlovian aversive conditioning which lead to behavioral suppression rather than locomotory activation. Several studies have been able to show increases in DA concentrations within the ventral striatum as a consequence of such conditioning (Young et al., 1993; Besson and Louilot, 1995; Saulskaya and Marsden, 1995) although so far none have addressed whether the changes are related to specific accumbens subregions. A related study by Wilkinson et al. (1998) has investigated parallel changes during acquisition and extinction of aversive CS conditioning in rats of DA in the nucleus accumbens and medial prefrontal cortex. This study showed greater changes initially during acquisition in the medial prefrontal cortex, but then subsequently greater responses in the nucleus accumbens that appeared to map onto the changes in behavioral freezing seen as a consequence of such conditioning and extinction in these rats.

Of particular interest is the study by Young et al. (1998) which utilized sensory preconditioning. Initially, dialysis showed increased overflow of ventral striatal DA in response to a pairing of motivationally neutral visual and auditory stimuli. Then, one of the stimuli (e.g. tone) was paired with an aversive foot-shock, after which the response to tone and light was measured separately, in the absence of the shock. The impressive finding was that accumbens DA was elevated in response to the light when it had been previously paired with the tone, but not when it had been unpaired. This suggests that associative conditioning can lead to an increase in accumbens DA in a situation in which it is difficult to explain simply in terms of an orientational behavioral response to the light (although that possibility cannot be entirely excluded). An earlier study had in fact shown that the latent inhibition of aversive conditioning to a tone by its previous nonreinforced exposure to the animals, produced parallel reductions in extracellular accumbens DA (Young et al., 1993).

A recent study by Levita et al. (2002), using a sophisticated design for analyzing aversive learning, was unable to show any evidence for DA overflow in the nucleus accumbens core region during aversive CS conditioning. These authors, in reviewing

previous and more recent (e.g. Pezze et al., 2001) studies on this theme were able to highlight several procedural difficulties associated with the earlier studies sufficient to cast doubt on the notion that increases in DA levels in the nucleus accumbens are reliably obtained; these authors attempt to resolve some of these discrepancies by postulating that specific regions of the core may be implicated in the processing of aversive CSs, consistent to some extent with the electrophysiological data of Schultz reviewed here.

In general, it appears from studies of in vivo monitoring of DA by dialysis and other neurochemical techniques that DA release occurs during aversive, as well as appetitive settings, in certain circumstances. This is in line with another evidence from a variety of sources, indicating that DA turnover is increased tonically during stress, particularly in the medial prefrontal cortex, and in the striatal regions, such as the nucleus accumbens shell (Kalivas and Duffy, 1995). However, the evidence that these changes are directly implicated in the associative learning process is at present less convincing.

2.3. PSYCHOPHARMACOLOGICAL EVIDENCE

The use of a direct intervention, whether a lesion, a drug treatment, a conditional knock-down of a gene, or any other treatment, is one of the key methods for determining whether a structure or a neurotransmitter pathway, such as DA, is actually necessary for learning, as distinct from correlated with it, as can be ascertained from neurochemical or electrophysiological monitoring in parallel with behavioral learning. The classical approach for demonstrating a selective role for a neural structure or neurotransmitter pathway in associative learning is to show a specific effect of a given manipulation on acquisition, but not preestablished performance. This pattern of results would normally indicate that the manipulation has probably interfered with processes of associative learning rather than other nonassociative processes inevitably confounded with learning, including perception, attention, motivation and motor function. As a facilitation of learning is always a more impressive demonstration than its impairment, this provides the gold standard for interpretations of specific effects on learning. Of course, if a given manipulation affects performance as well as learning, then parsimony dictates that a nonassociative effect can account for both sets of findings. Alternatively, it is plausible that the manipulation separately interferes with both associative and nonassociative factors; however, for that interpretation to hold, it might be expected that the effect on learning would be quantitatively greater than any effect on performance. With these general points in mind, it is evident in reviewing the experimental literature that it is still quite difficult to find consistent evidence for a specific role for brain DA systems in learning that matches predictions from the electrophysiological evidence.

The fact that the release of DA can function as a reinforcing event, as inferred for example, from studies on the self-administration of DA-ergic drugs (see Chapter 6 by Di Chiara), suggests that it has some role in learning, even if it is only by contributing to the affective representation of the unconditioned reinforcer. This conclusion is also generally consistent with the evidence reviewed above on neurochemical monitoring. In general, psychopharmacological evidence showing a specific role for DA in learning is rather limited because drugs have generally been administered to animals exhibiting steady-state performance. There is no doubt that drugs, such as amphetamine, as well as more specific DA agonists and antagonists, have profound effects on performance in a variety of appetitive and aversive situations. However, such effects potentially confound an analysis of their possible effects on learning. It is clear, for example that the acquisition

of responding with conditioned reinforcers is potentiated by amphetamine-like drugs via DA-dependent mechanisms of the nucleus accumbens that include the shell region (Robbins et al., 1989; Parkinson et al., 1999; review by Sutton and Beninger, 1999). However, it is more dubious that this potentiation reflects a facilitation of associative learning rather than response rate-increasing effects of these drugs. Thus, neither mesolimbic DA depletion achieved via 6-OHDA lesions of the nucleus accumbens (Taylor and Robbins, 1986) nor intraaccumbens infusions of selective DA D1 or D2 receptor antagonists (Wolterinck et al., 1993) in themselves appear to impair the acquisition of a new instrumental response for a conditioned reinforcer, as distinct from blocking the potentiative effects of d-amphetamine.

There are analogous impairments in the acquisition of active avoidance behavior produced by neuroleptic drugs and the role of negative conditioned reinforcers (see Blackburn et al., 1992). In an early experiment (Beninger et al., 1980), systemic pimozide was shown to have its normal disruptive effect on signaled avoidance behavior. However, when the capacity of the signal to act as a fear signal was assessed independently, animals receiving pimozide during the Pavlovian conditioning phase nevertheless exhibited normal levels of conditioned suppression to the CS on a food-reinforced baseline, thus demonstrating intact associative fear conditioning.

Other experiments by Beninger and Phillips (1980) focused on appetitive associative learning and showed that systemic injections of the DA-receptor antagonist pimozide may have impaired the acquisition by rats, of an association between a specific CS and food presentation. When the rats were subsequently tested in the undrugged state in a situation requiring the new learning of a response to produce the CS as a conditioned reinforcer, this effect was attenuated in the rats previously treated with pimozide. However, it is always difficult to be sure that some unmeasured effects of the drug (e.g. changes in eating rate) did not interfere with the associative process indirectly. As with effects on active avoidance acquisition (see Blackburn et al., 1992) it is difficult to be sure that the drug effect does not simply reflect an effect on motor performance (see also Salamone, 1994).

On the other hand, in the investigation of systemic effects of a low and a high dose of the D2/D3 receptor agonist quinpirole, Nader and LeDoux (1999) have recently employed an inactive response (defensive freezing) and a sophisticated design which separated basic effects on associative learning and sensory processing via a comparison of groups of rats subjected to second order fear learning or sensory preconditioning. They found that, when quinpirole was administered prior to the CS1–CS2 pairing stage, there was a subsequent block of aversively-motivated freezing behavior in the quinpirole-treated rats, suggesting an attenuation of the retrieval of the fear associated with the CS that is hypothetically mediated via a reduction of DA neurotransmission through D1-like post-synaptic mechanisms in unspecified anatomical structures. The lack of effect on sensory preconditioning is not consistent with the demonstration by Young et al. (1999) of an elevation of nucleus accumbens DA during CS1–CS2 sensory preconditioning.

Experiments using the neurotoxin 6-OHDA to produce selective and profound depletions of DA in certain regions, such as the nucleus accumbens or caudate-putamen, have also been shown to impair instrumental visual discrimination learning (Evenden et al., 1989; Robbins et al., 1990) and more recently, discriminated approach behavior that is largely controlled by Pavlovian contingencies ('autoshaping') (Dalley et al., 2002; Parkinson et al., 2002). However, in most of these experiments impairments produced by such DA-depleting lesions were also seen in rats trained earlier, although the deficits were not necessarily as great as those observed during acquisition (e.g. Parkinson et al.,

2002). In the appetitive autoshaping paradigm, intra-accumbens infusions of the D1/D2 receptor antagonist alpha-flupenthixol did apparently impair discrimination to a much greater extent in acquisition than performance (DiCiano et al., 2002).

Potentially confounding effects on learning of motivational or attentional changes resulting from pretraining treatments are minimized by post-training administration. In fact, post-trial administration of amphetamine under certain conditions can subsequently enhance memory when retention is tested several days later, in both appetitively and aversively-motivated tasks (e.g. Krivanek and McGaugh, 1969). For a while, it was thought that such actions were largely mediated peripherally, as the 'memory-enhancing' effects could be blocked by adrenalectomy (Martinez et al., 1980). However, experiments by Carr and White (1984) and others have shown that a central, probably caudate, site could at least contribute to this enhancement of memory consolidation. In further extensions of the work, intra-caudate administration of the D2/D3 agonist quinpirole produced improved retention of a conditioned suppression task. In theory, such effects could still be explained if, for example, the drug directly strengthened the processing of the US, e.g. perhaps increasing the subjective sensation for the animal of the shock. However, several ingenious experiments have excluded this possible interpretation. For example, White and Viaud (1991) varied not only the site of infusion within the caudate but also the sensory modality of the CS. When the DA-ergic agent was infused into that anatomical region of the rat caudate-putamen known to receive input from cortical visual areas, it subsequently enhanced learning of the visually cued learning; the same was true of the enhancement of olfactory cued aversive learning. Therefore, the enhancement only occurred when the DA agonist interacted with that region of the striatum processing the CS, and also only affected the response to this stimulus if it had been contingently related to the shock US, thus strongly supporting some specific modulation of post-trial associative processing.

This technique of post-trial manipulation of the modulation by DA of memory consolidation processes has now been extended to forms of memory mediated by other terminal domains. Packard and White (1991) showed that post-trial administration of d-amphetamine, the D2/3 agonist quinpirole, or the D1 receptor agonist SKF-38393 to the caudate (but not the hippocampus) all enhanced subsequent retention of an appetitive 'win-stay' task carried out in a radial maze, whereas similar administrations to the hippocampus (but not the caudate) enhanced learning of a 'win-shift' procedure in the same apparatus. These effects seem very difficult to explain simply in terms of general performance-altering effects of the drug.

This possible role for DA in modulating the consolidation of longer term spatial memories known to depend on the hippocampus has been extended to the nucleus accumbens. Ploeger et al. (1994) were initially able to show that intra-accumbens haloperidol impaired acquisition of the Morris water maze escape task, but a yet more significant demonstration is that of Setlow and McGaugh (1998) with immediate post-trial administration of the DA D2 receptor antagonist sulpiride, leading to a retention deficit two days later. The delayed infusions or immediate post-trial infusions of sulpiride, using an externally cued version of the task failed to affect retention, suggesting a specific effect on the consolidation of long term spatial memory. These authors speculate on the basis of other results that these DA-dependent processes of the nucleus accumbens are only implicated in consolidation of the memory and not in its storage. The consolidation of long term spatial memory, however, is unlikely only to involve the ventral and not the dorsal striatum. In a second experiment, Setlow and McGaugh (1999) reported results

obtained following post-trial sulpiride infusions into the posteroventral caudate-putamen, which they interpreted to reflect memory for procedural aspects of the task. The sulpiride-treated rats spent less time swimming in the vicinity of the earlier trained platform, although they reached the platform location with a normal latency. Thus DA-ergic processes appear to modulate several aspects of memory associated with this task in different regions of the striatum that are in receipt of different limbic-cortical afferents. These DA-ergic influences may also include projections to such limbic structures themselves. Thus, the above results have been extended by the demonstration that post-trial infusions of amphetamine into the amygdala modulate retention of both a cued and a spatial version of the Morris water maze (Packard et al., 1994), potentially via DA-ergic mechanisms. A more recent study has also shown that post-trial, systemic DA D2/D3 receptor agonist injections enhance consolidation of spatial learning in the Morris water-maze (Setlow and McGaugh, 2000). These data are consistent with the evidence that manipulation of hippocampal DA affects the induction of hippocampal LTP (Otmakhova and Lisman, 1996).

In parallel with experiments reviewed that indicate DA-ergic modulation of hippocampal processing, another set of experiments has analyzed the effects of specific manipulations of DA-ergic transmission on the consolidation of stimulus-reward learning or 'emotional memory' associated in particular with the amygdala. Hitchcott et al. (1997a) initially found that intra-amygdaloid, post-trial amphetamine enhanced the acquisition of a discriminative approach response to sucrose solution. They then examined effects of the DA receptor agonists SKF-398393 (D1), quinpirole and 7-OH-DPAT (D2/D3) (Hitchcott et al., 1997b). Significant enhancement of discriminative approach was found at certain doses of 7-OH-DPAT. However, the precise locus of this effect within the amygdala (e.g. central nucleus or basolateral amygdala) is somewhat unclear, although presumably the greater density of D2/D3 receptors in the central nucleus implicates that structure, possibly through its involvement in Pavlovian appetitive learning (Parkinson et al., 2000).

3. ROLE OF DA IN ATTENTION

3.1. PSYCHOPHARMACOLOGICAL EVIDENCE

The earliest reports of effects of unilateral striatal DA depletion in the rat on rotational behavior in response to DA-ergic drugs were soon followed by observations of behavioral symptoms interpreted as forms of attentional or sensori-motor 'neglect'-failures to orient accurately to exteroceptive stimuli such as von Frey hairs (Ungerstedt, 1971; Marshall and Teitelbaum, 1977). Studies utilizing primates (Schneider, 1990; Annett et al., 1992) have found analogous symptoms. Detailed analysis in rats of the 'neglect' syndrome, has shown that it is mainly attributable to DA depletion from the dorsal striatum (caudate-putamen) and that it may result from impairments in such processes as the preparatory readiness of orienting responses (Carli et al., 1985; see review by Robbins and Brown, 1990; Ward and Brown, 1996).

Three other main test paradigms have been used that appear to expose the possible attentional dysfunction following manipulations of DA-ergic function: latent inhibition; prepulse inhibition and continuous performance (the five-choice serial reaction time task) – all notable for their correspondence to similar tests for human subjects. Curiously, the main emphasis of these investigations has been on mesolimbic rather than mesostriatal systems.

Latent inhibition is the retardation of conditioning that occurs following nonreinforced pre-exposures of the CS (Mackintosh, 1983). This has been interpreted as an attentional effect, although other possible interpretations of LI exist (see below). LI is impaired following systemic doses of d-amphetamine, so that learning is actually facilitated in the pre-exposed condition. These effects, however, are apparently restricted to the learning rather than the pre-exposure stages of the test, to the use of low and intermediate doses of the drug, and are more readily obtained following chronic administration (Weiner et al., 1984, 1987; Weiner, 1990). Similar effects are also much more difficult to obtain following treatment with DA receptor agonists such as apomorphine (Moser et al., 2000). Thus, from the perspective of DA-ergic function, more impressive evidence derives from effects of systemically administered DA receptor antagonists, which consistently facilitate latent inhibition in rats (Moser et al., 2000).

The position in humans though, is more equivocal and may reflect the difficulty of being sure that what is being studied as LI in rats and humans necessarily reflects the same processes. One study (Williams et al., 1997) has reported the expected enhancement of latent inhibition using a visual task following low i.v. doses of haloperidol. However, the same group have also now reported the opposite result in young volunteers with an auditory paradigm – namely impaired latent inhibition (Williams et al., 1998). This is a particularly important result, as schizophrenics naive to neuroleptic medication were shown not to have the usual deficits in LI associated with chronic (and medicated) schizophrenia. The implications are that DA receptor antagonism may sometimes impair latent inhibition, possibly via attentional factors. Thus, the deficits in LI in schizophrenia may arise, at least in part, as side-effects of such medication.

Original theories focused on the likely role of the nucleus accumbens in mediating effects of DA-ergic drugs on latent inhibition, but this conclusion remains controversial. For example, Killcross and Robbins (1993) found that intra-accumbens infusions of d-amphetamine, while impairing aversive conditioning (measured in terms of conditioned suppression) per se, did not differentially affect pre-exposed versus non-preexposed stimuli, in a within-subject design. Systemic treatments with either d-amphetamine or a neuroleptic drug (alpha-flupenthixol) did produce the usual deficit and enhancement, respectively. However, these were later shown to depend on apparent drug-reinforcer interactions (Killcross et al., 1994a,b). Amphetamine appeared to enhance conditioning by enhancing the impact of the reinforcers (electric shock or sucrose). By contrast, the neuroleptic had the opposite type of effect on the reinforcers, possibly accounting for its contrasting effect on latent inhibition. Consistent with the findings of Killcross and Robbins (1993), Ellenbroek et al. (1997) found impaired latent inhibition following dorsal rather than ventral striatal infusions of amphetamine, but they employed a taste-aversion procedure for assessing latent inhibition.

Other data have been interpreted to indicate that the nucleus accumbens is an important site for LI. In the original experiment Solomon and Staton (1982) demonstrated impaired latent inhibition following chronic ventral rather than dorsal striatal infusions of amphetamine, though they employed an active avoidance rather than a conditioned suppression procedure which may have been more compatible with psychomotor stimulant effects. Gray et al. (1995) reported in a review data indicating that mesolimbic DA depletion appeared to facilitate latent inhibition, also consistent with the results of microdialysis studies and the effects of DA receptor antagonists, described above (Gray et al., 1995).

At this stage it would be unwise to conclude that the role of DA in LI has been fully elucidated. It appears that the effects of intra-accumbens manipulations on latent inhibition may depend on the chronicity of treatment, the precise nature of the behavioral paradigm employed for measuring latent inhibition, and possible side-effects of the drug on the impact of the reinforcer. An over-riding consideration is that effects on LI may not derive from modulation of attentional processes but instead reflect altered processing of the unconditioned reinforcer, or as has been argued previously (Killcross et al., 1994a,b), memory retrieval processes based on contextual processing. Specifically, drugs such as amphetamine, which enhance the effectiveness of the reinforcer, might increase the difference in context between the pre-exposure and testing stages of the LI paradigm, which would by itself attenuate LI. DA receptor antagonists could be expected to have the opposite effect.

A distinct form of attention is probably exemplified by the phenomenon of pre-pulse inhibition (PPI), in which a less intense surrogate stimulus reduces the magnitude of the acoustic startle response to an intense loud noise (Braff and Geyer, 1992) – paralleling its apparent action to protect against the reduction in extracellular DA levels produced by such a startle stimulus (Humby et al., 1996). DA-dependent mechanisms of the nucleus accumbens are certainly implicated in this response, although deficits in this 'sensori-motor gating' process are produced by both DA D2 receptor agonists and antagonists (Swerdlow et al., 1994; Geyer et al., 2001). Intriguingly, it appears that whereas LI is more susceptible to disruption by amphetamine than apomorphine, the reverse is true for PPI. Therefore the exact pre- and post-synaptic DA receptor mechanisms that modulate PPI and LI, and possibly also their anatomical location, may be distinct. Recent studies with transgenically modified mice have confirmed a possibly key role for the DA D2, rather than the D3 or D4 receptor in PPI (Ralph et al., 1999). There are also considerable strain differences in the role of D2 receptors within the nucleus accumbens for the PPI response in the rat (Kinney et al., 1999). Wan and Swerdlow (1998) have further provided evidence that this form of 'sensori-motor gating' is mediated by DA–glutamate interactions within both the core and shell subregions of the nucleus accumbens.

Despite their subtle differences in pharmacology (Moser et al., 2000; Geyer et al., 2001) there have been few direct comparisons of PPI and LI; one such was made in a study that investigated the responses of rats reared in social isolation, which have elevated levels of extracellular striatal DA (Wilkinson et al., 1994). Social isolation impaired prepulse inhibition, but not latent inhibition. The prepulse inhibition deficit is of considerable interest, not least because of possible relevance in schizophrenia (Braff et al., 2001), but also to illustrate how descending forebrain influences, including the nucleus accumbens, modulate the 'tone' of a set of reflexes organized in the brain stem. One interpretation of the effects of fluctuations of DA activity on PPI is that alteration of this 'tone' may be a consequence of reinforcing events that lead to changes in DA-ergic function.

Possible effects of DA on attentional functions have also been investigated using a number of tasks which require animals to detect signals over a protracted period of stable performance. One such paradigm, the five-choice serial reaction time task, was developed by analogy from human studies (see Robbins, 2002 for review). Rats are required to detect brief visual stimuli presented randomly in one of five locations in a specially-designed apparatus. The temporal predictability of the stimuli as well their detectability (via manipulations of stimulus illumination and duration) can also be varied. Initial experiments focused on neuropharmacological manipulations of mesolimbic DA function. Depletion of mesolimbic DA using 6-OHDA had little effect on the accuracy of stimulus

detection under any experimental conditions. However, the latency of responding was lengthened, errors of omission increased, and premature responses reduced in certain conditions (Cole and Robbins, 1989). This pattern of effects is consistent with effects of mesolimbic DA on the invigoration of behavior, perhaps via motivational influences, rather than a disruption of attention. Complementary effects were obtained when d-amphetamine was infused into the nucleus accumbens; again there were no effects on choice accuracy, but premature responses were greatly increased in frequency (Cole and Robbins, 1987).

There have now been parallel studies of 6-OHDA-induced lesions of the mesostriatal and mesocortical DA systems (Robbins et al., 1998; Baunez and Robbins, 1999). Both studies produced results that were different from those of mesolimbic DA loss, in that there were impairments in choice accuracy when the visual stimuli were presented in a temporally unpredictable manner. The impaired choice accuracy resulting from mesostriatal DA depletion was found in the context of many other behavioral deficits, including slowed responding and large increases in response latency (similar to those seen following mesolimbic DA loss). However, despite these effects, no deficits in accuracy were observed under baseline conditions. The selective disruption produced by the variable intertrial intervals may be related to the basic impairments in the readiness to respond described in earlier studies on simple and choice reaction time (Brown and Robbins, 1991).

Following mesocortical DA loss there were few other impairments in this task, but the specific deficit in accuracy might possibly have been attributable to the almost unavoidable depletion of noradrenaline from the prefrontal cortex following such 6-OHDA lesions. Further specific evidence for a role of DA receptors in attentional accuracy is provided by recent results following infusion of specific DA receptor agonist and antagonists into the prefrontal cortex. Intra-cortical infusions of the D1 DA receptor antagonist SCH-23390, but not the DA D2 receptor anatagonist sulpiride, produced selective impairments in the accuracy of responding, whereas similar infusions of the partial D1 receptor agonist SKF-38393 actually improved choice accuracy under some conditions (Granon et al., 2000).

One of the difficulties of assessing the role of DA in attention derives from the diverse nature of attentional processes, which include selective attention, divided attention, sustained attention and vigilance. Advances in cognitive neuroscience increasingly indicate that such processes are probably subserved by distinct neural systems, beginning for example, with Posner's 'anterior' and 'posterior' cortical attentional systems (Posner and Petersen, 1990). The prefrontal cortex is especially associated with the former type of function, including 'attention to action', and performance on tests such as the Wisconsin Card Sort Test (WCST). An influential hypothesis has linked 'hypofrontality', including mesofrontal DA underactivity in the human prefrontal cortex, to the deficits shown by schizophrenic patients on this task in the context of functional neuroimaging studies (Weinberger et al., 1988). Consequently, recent studies of effects of frontal DA loss on analoges of such tests in monkeys are of considerable significance (Roberts et al., 1994; Crofts et al., 2001). Damage to the marmoset lateral prefrontal cortex, made with an excitotoxic amino acid, impairs the ability of the monkeys to shift attentional set from one perceptual dimension to another, for a complex stimulus consisting of at least two stimulus dimensions – a so-called 'extra-dimensional shift' (see Fig. 1; Dias et al., 1996). However, such damage fails to affect the learning of such discriminations, the maintenance of attentional set within stimulus dimensions, or reversal learning (which is, however,

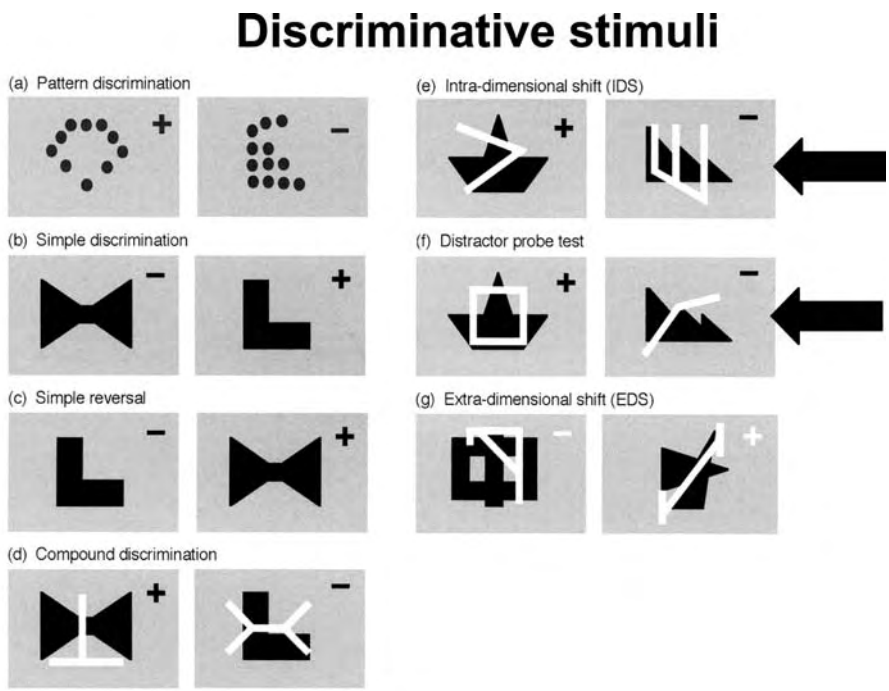

Fig. 1. Discriminative stimuli used in tests of intra- and extra-dimensional shifts of attention in marmosets with lesions of the prefrontal cortex (PFC) or 6-OHDA-induced depletion of DA from PFC. Plus and minus signs indicate reinforced and non-reinforced discriminanda, respectively. The arrows indicate those stages especially sensitive to PFC DA depletion as shown by Crofts et al. (2001). Adapted from Crofts et al. (2001).

impaired by orbitofrontal lesions) (Dias et al., 1996). By contrast, 6-OHDA-induced lesions of the marmoset prefrontal cortex, which led to considerable DA (as well as significant noradrenaline) depletions throughout the various sectors of the prefrontal cortex, produced what was initially an unexpected pattern of findings: marmosets with prefrontal DA loss performed the extra-dimensional shift faster than normal monkeys (Roberts et al., 1994). One possible neural mechanism underlying this surprising result was the discovery from microdialysis studies of a parallel upregulation of striatal DA function in the same animals. (A reciprocity between the regulation of subcortical and cortical DA systems was not at all unprecedented from previous literature, beginning with Carter and Pycock, 1980).

Further studies also probed the possible psychological concomitants of the anomalous faster set-shifting. One possibility was that the more rapid shifting reflected an instability of the dominant set. The monkeys in the Roberts et al. (1994) experiment had received extensive training prior to surgery, which might have masked such attentional lability. Therefore, in a new experiment (Crofts et al., 2001), marmosets received 6-OHDA lesions of the prefrontal cortex after relatively minimal training. These animals were much less able to take advantage of successive intra-dimensional shifts to improve their performance, suggesting that the prefrontal DA-depleted monkeys were less able to focus responding successfully on a single stimulus dimension (e.g. 'shape') (Fig. 2). Particularly revealing was the additional observation when the irrelevant, background stimuli were

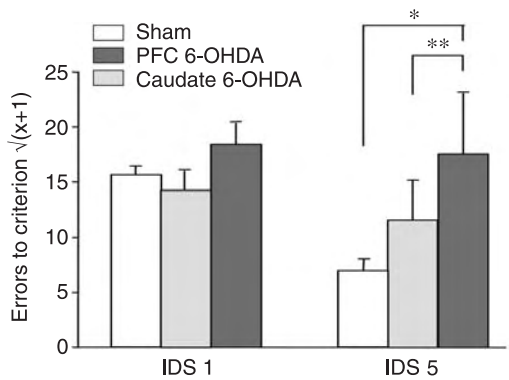

Fig. 2. Mean number of errors (±SEM) made by control ($n=7$), 6-OHDA caudate ($n=8$) and 6-OHDA PFC ($n=7$) lesioned monkeys on the first and final discrimination in a series of five intra-dimensional shifts (IDS) (stimuli shown in Fig. 1). *$p < 0.05$, **$p < 0.01$. Adapted from Crofts et al. (2001).

changed, which caused much more disruption in monkeys with prefrontal DA loss, than other groups of animals undergoing sham surgery or depletion of DA from the head of the caudate nucleus, suggestive of enhanced 'distractibility' in the prefrontal DA-depleted animals (Fig. 3). Of equivalent interest was the finding of reduced distractibility, relative to controls, of marmosets with striatal DA loss (Fig. 3). This again serves to underline the reciprocal nature of cortical and striatal DA systems; clearly the relevant balance in activity between the two systems serves to regulate the range of discriminative stimuli to which the relevant fronto-striatal system is responsive. The Crofts et al. (2001) study was further able to reproduce the enhanced extra-dimensional shifting from the Roberts et al. (1994) study. However, this effect was shown to depend on the relative strength of control exerted by the two perceptual dimensions ('shape' and superimposed 'line', respectively).

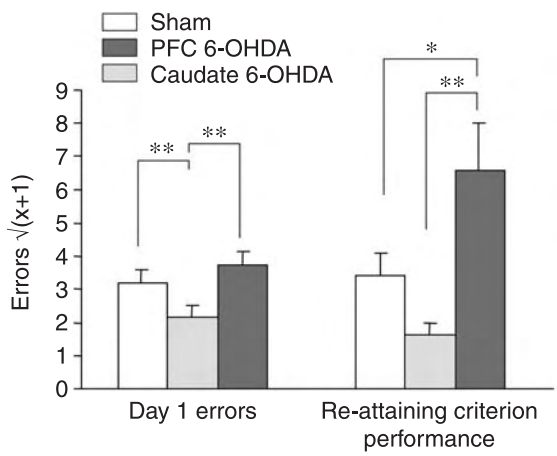

Fig. 3. Mean number of errors (±SEM) made by control ($n=7$), 6-OHDA caudate ($n=8$) and 6-OHDA PFC ($n=7$) lesioned monkeys on the first session of the distractor probe test (see Fig. 1) and before reattaining criterion levels of performance. *$p < 0.05$, **$p < 0.01$. Adapted from Crofts et al. (2001).

Specifically, the prefrontal DA depleted marmosets were faster to shift from 'lines' to 'shapes', but not vice versa. Other observations also supported the hypothesis that DA depletion was particularly implicated when attentional control had to be exerted to enable responding to stimuli that generally lack salience for the animal. Crofts et al. (2001) hypothesize that prefrontal DA is implicated in 'top-down' rather than 'bottom-up' attentional processing. This hypothesis is not at all incompatible with the notion that the prefrontal DA systems are 'engaged' under certain circumstances in order to optimize performance (e.g. Granon et al., 2000). The results have further theoretical implications for attempts to model the functioning of the prefrontal DA systems (e.g. Dustrewicz et al., 2000). The experimental observations are also relevant to a range of clinical pathologies, ranging from Parkinson's disease to schizophrenia and ADHD, especially given the observations of reduced DA function in the prefrontal cortex of ADHD individuals (Ernst et al., 1998).

Additional studies of striatal DA loss in the marmoset also show that this fails to impair extra-dimensional shift performance as affected by prefrontal cortical lesions (Collins et al., 2000). However, the striatal DA loss does produce an impairment in shifting between stimulus dimensions when the requirement is to shift back towards a previously irrelevant dimension, which is possibly relevant to impaired performance of human patients with Parkinson's disease of shifting between two well-established 'task sets' (Cools et al., 2001).

3.2. MODELS OF ATTENTION DEFICIT AND HYPERACTIVITY DEFICIT (ADHD)

The clinical entity of ADHD and the therapeutic effects of methylphenidate (Ritalin) and amphetamine have encouraged animal models of this syndrome especially for understanding the apparently paradoxical effects of psychomotor stimulants in reducing high levels of locomotor activity (see Robbins and Sahakian, 1979; Seiden et al., 1989). Recent approaches have capitalized on genetic technology. So, for example, the DA transporter knockout (DAT) mouse has elevated DA-ergic tone, is hyperactive and also exhibits deficits in tests of spatial memory (Gainetdinov et al., 1999). Methylphenidate was shown to antagonize this hyperactivity, although possible beneficial actions on spatial or other forms of cognition were not investigated. Possible mechanisms of action of methylphenidate in this model, as well as in ADHD itself, are unclear. They could include an action on another neurotransmitter system such as the central serotoninergic systems originating in the raphé nuclei (Gainetdinov et al., 1999). Some support for this view was provided by the observation that hyperactivity in the DAT knockout mice was antagonized by chronic treatment with the selective serotonin reuptake blocker fluoxetine, although whether this type of mechanism is responsible for the effects of Ritalin is unknown (see Solanto et al., 2001).

4. WORKING MEMORY

In the neuroscience literature the construct of working memory generally refers to the capacity to hold information 'on-line' for a period during which the eliciting stimulus is no longer present. According to Goldman-Rakic (1987), therefore, this form of working memory has a crucial role in the intermediate stages of stimulus processing, to provide

input to brain structures that form representations of the world. A somewhat different perspective is provided by Olton's observations of rats performing radial maze tasks according either to recently acquired information or to permanent, long-lasting 'response rules' (Olton et al., 1979). Thus, within a single set of trials, perhaps with interpolated delays, rats will learn systematically not to return to recently baited arms within the maze, a 'win-shift' tendency denoted as 'working memory'. On the other hand, rats will consistently choose arms always baited with food over repeated test sessions in preference to arms not reliably associated with food ('reference memory').

These concepts are related to the more elaborate concept of working memory in human cognition of Baddeley (1986), which includes different two distinct short term memory stores (the 'articulatory loop', a form of sub-vocal rehearsal mechanism, and a 'visuospatial sketchpad', a short-term memory buffer for visuospatial imagery). Both these stores hold stimuli 'on-line' for further processing. An additional, and more controversial, feature of Baddeley's scheme is the postulate of a 'central executive' system which co-ordinates processing between the various satellite systems. This executive role is commonly equated to the functioning of the prefrontal cortex although such a simple mapping of psychological processes onto anatomical structures is not particularly helpful. The 'central executive' system of Baddeley (1986) has much in common with another model of the frontal lobe functioning termed the 'supervisory attentional system', in which control over instrumental choice behavior is exerted through 'attention to action' (Shallice, 1988). This concept is particularly relevant to paradigms such as the spatial delayed response task in which there are other cognitive requirements besides 'holding stimuli on-line', for example the inhibition of repeated responses to pre-potent stimuli (Diamond, 1996). The precise relationship of concepts of working memory across human and animal research can be a source of difficulty of interpretation, and has been much debated (see Roberts et al., 1998). This debate is relevant to the interpretation of behavioral processes required for tests of 'working memory' function in experimental animals such as the delayed response task, used mainly for primates and the delayed alternation test, which has analogies with the radial arm maze paradigm of Olton described above and is more often used when testing rodents.

4.1. PSYCHOPHARMACOLOGICAL EVIDENCE

Pharmacological manipulation of DA, within mesostriatal, as well as mesofrontal domains, has profound effects on performance in spatial working memory tasks in both rodents and monkeys. Early work (reviewed by LeMoal and Simon, 1991) demonstrated that 6-OHDA-induced lesions of the meso-accumbens or meso-striatal, as well as the meso-cortical DA projections led to impaired delayed alternation performance in rats. However, there is a question of whether the capacity to hold 'on-line' the location of the previous goal or choice response has been impaired or whether other behavioral capacities, such as the inhibition that is normally required for the spontaneous alternation of choices is disrupted, leading to perseverative responding.

Brozoski et al. (1979) performed a landmark study on the role of PFC DA in working memory function in monkeys. These investigators used a delayed response type procedure to show that 6-OHDA-induced depletion of DA in the principal sulcus of the dorsolateral prefrontal cortex in macaques produced an impairment as profound as ablation by aspiration of the region itself. In contrast, depletion of either noradrenaline or 5-HT in the prefrontal cortex had little effect. Convincing evidence for a specific role of DA came from

additional evidence that the deficits could be abolished by systemic treatment with drugs such as apomorphine and L-Dopa.

Goldman-Rakic and collaborators extended these classical findings by employing a 'delayed saccade' procedure which is impossible to solve by 'mediating' responses, as monkeys have to hold fixation of a central spot before making an eye-movement shifting gaze to the location of a brief visual stimulus presented a few seconds earlier. Selective disruptions in the accuracy of the 'memory saccades' were produced by iontophoretic application into the PFC of doses of DA D1, but not D2, receptor antagonists (e.g. Sawaguchi et al., 1991). These findings have been supported by experiments with a delayed response procedure in marmosets which removed the possibility of mediating responses by distracting the animal to the rear of the testing chamber during the delay period (Roberts et al., 1994). Once again, DA depletion from the PFC was found to impair the acquisition of a spatial delayed response task, though not to quite the same extent as an excitotoxic lesion of most of the PFC itself. A key finding from a further study (Collins et al., 1998) was the sparing, following mesocortical DA depletion, of the capacity to self-order responses without perseveration (which was, in comparison, markedly impaired by excitotoxic lesions). Thus DA apparently modulated mnemonic functions associated with the working memory task rather than the 'executive' operations of producing the optimal response sequence.

In monkeys, investigators have been rather slow to test the hypothesis of possible striatal involvement in working memory function, as measured by delayed response performance. Arnsten et al. (1994) have shown beneficial effects of DA D2 receptor agonists in aged macaques, suggesting a possible striatal role in view of the much greater density of D2-like receptors in this region as compared with the prefrontal cortex. A rather different sort of study by Castner et al. (2000) showed that chronic treatment of monkeys with D2 receptor blocking antipsychotic agents led to working memory deficits in the spatial delayed response task that were dramatically ameliorated by the short-term co-administration of the D1 receptor agonist ABT 431 (Fig. 4). This is consistent with evidence that such chronic treatment produces a down-regulation of D1 receptors in the PFC via mechanisms requiring further elucidation. The results are fully consistent with an important role for PFC D1 receptors in working memory and suggest that pharmacological modulation of the D1 receptor can produce long-lasting changes,

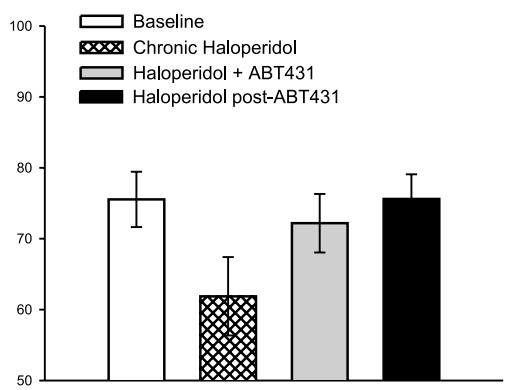

Fig. 4. Spatial delayed performance in rhesus monkeys following chronic treatment with the D2 receptor antagonist haloperidol, and the short-term remediation produced by acute treatment with the D1 receptor agonist ABT 431, as well as the longer term benefit produced by that acute treatment. Adapted from Castner et al. (2000).

possibly via the cAMP cascade, in the functional circuitry underlying working memory, with potentially important therapeutic implications.

Collins et al. (2000) recently produced selective lesions of the caudate DA system, using infusions of 6-OHDA in the terminal fields, and found direct evidence for a delayed response deficit following striatal DA loss. These findings are consistent with the earlier findings of Schneider (1990), following treatment with the neurotoxin MPTP, as a model of Parkinson's disease, although MPTP has effects that are neither restricted to the striatum, nor to DA. The precise nature of the deficits in spatial delayed response following striatal DA loss are not known. Taylor et al. (1990) found that the treatment of monkeys with MPTP also impaired their capacity to inhibit reaching through a transparent barrier in an Object Retrieval task, rather than making a more effective 'detour reach'. This paradigm clearly implicates to a much greater extent, response inhibitory rather than working memory functions, and so this observation is not entirely consistent with the findings of Collins et al. (1998) referred to above, which failed to find specific effects of prefrontal DA depletion on response inhibitory functions. However, it is unclear as to what extent the deficit in object retrieval found by Taylor et al. depends on striatal or cortical DA loss. A future challenge will be to delineate with greater precision the relative contributions of prefrontal and striatal DA to spatial delayed response performance in monkeys.

Seamans and colleagues (e.g. Floresco et al., 1996; Seamans et al., 1998; Floresco and Phillips, 2001), in a succession of elegant studies, have shown how the prefrontal and ventral striatal DA systems have different roles in the mediation of foraging performance by rats in a number of radial eight-armed maze tests (see Fig. 5). For example, microinjections of the D1 receptor antagonist SCH-23390 (but not the D2 receptor antagonist, sulpiride) into the prelimbic region of the PFC disrupted performance of a delayed version of the task (similar to that used by Packard and White, 1991) in which spatial information acquired during a training phase was used 30 min later prospectively to guide responses, but had no effect on choice performance in the maze in the absence of delay. These effects were further shown to depend on the modulation of hippocampal inputs to the PFC by the use of crossed, asymmetrical manipulations. The authors' hypothesis was that the information may be held within the hippocampus until required for formulating a subsequent plan to guide action. Thus, DA hypothetically modulates a circuitry including the hippocampus at the level of the PFC which affects spatial working memory functioning, including its 'executive aspects'.

The contribution of the striatum to working memory was examined by these authors after intra-accumbens infusions of the DA receptor antagonist haloperidol (Floresco et al., 1996). This treatment did not affect performance on the delayed task described above, but did impair performance on the nondelayed, random foraging task in which rats have to retrieve within a single session 4 pellets from four different arms of the eight-armed maze. Haloperidol increased errors to both previously baited and nonbaited arms. Floresco et al. attributed the deficits to impairments in the processing of information from hippocampus to the nucleus accumbens normally implicated in the organization of foraging behavior.

4.2. EVOLVING INTERPRETATIONS OF THE ROLE OF THE PFC IN WORKING MEMORY: THE YERKES-DODSON PRINCIPLE

The effects on working memory processing shown following PFC infusions of a D1 receptor antagonist (Seamans et al., 1998) prior to retention testing are somewhat different

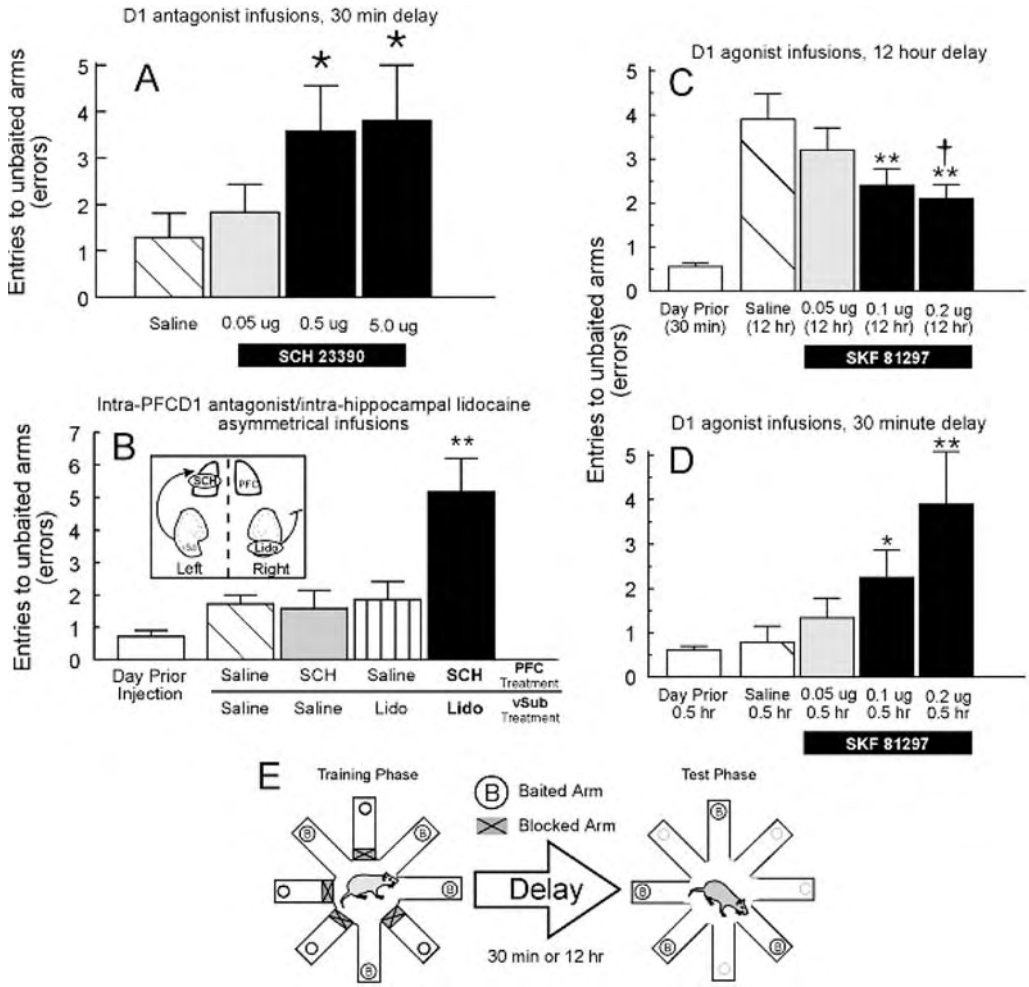

Fig. 5. **A**. Infusion of the D1 antagonist SCH 23390 prior to the test phase of the delayed spatial win shift (SWSh) task (black and grey bars) dose-dependently increased the number of errors committed during the test phase, compared to saline infusion (hatched bar). The delay between training and test phases was 30 min. **B**. Similarly, asymmetrical infusions entailing a unilateral inactivation of the ventral subiculum in combination of an infusion of SCH 23390 into the PFC (inset) also disrupted working memory (black bar). In contrast, unilateral inactivation of the ventral subiculum (striped bar), unilateral infusion of SCH 23390 into the PFC (grey bar), or saline in both regions (hatched bar) had no effect. The delay between training and test phases was 30 min. **C**. Insertion of an extended 12 h delay between training and test phases increased the number of errors made by rats treated with intra-PFC saline (hatched bar) compared to the day prior when a 30 min delay was inserted (open bar). However, infusions of the D1 agonist SKF 81297 (black and grey bars) prior to the test phase dose dependently improved performance, and reduced the number of errors. **D**. In contrast to **C**, when working memory is optimal at a 30 min delay, similar infusions of SKF 81297 prior to the test phase (black and grey bars) impaired performance; rats made significantly more errors during retrieval. **E**. The delayed SWSh task consists of a training and a test phase. During the training phase, 4 of 8 arms on a radial maze are randomly blocked, and the 4 remaining open arms are baited. Once the animal has retrieved the 4 pieces of food from the open arms, it is removed from the maze for a delay (either 30 min or 12 in the experiments described above). After the delay, the animal is placed back onto the maze for the test phase. The arms that were blocked previously are now open and baited. The rat must remember which arms were previously blocked and enter them to receive the food reward. All drugs were administered 5–10 min. Based on data reported in Floresco et al. (1996, 2001) and Seamans et al. (1998).

to other findings in rats using a delayed matching-to-position operant procedure (Broersen et al., 1995). The effects of the DA receptor antagonist were not clearly delay dependent in this latter study, unlike those of the muscarinic receptor antagonist scopolamine. The very different nature of the tasks and concepts of working memory, compared to those used by Seamans et al. (1998) may have contributed to this apparent discrepancy. Whereas the Seamans et al. study examined how modulation of DA function altered the choice based on retrieval of memories occurring some 30 min before, Broersen et al. investigated short term spatial memory requirements in terms of seconds, rather than minutes, of the spatial delayed alternation (and delayed response task). However, it is apparent that the Broersen et al. findings are probably more readily related to the spatial delayed response procedures used in monkeys. These contrasting effects are also of particular interest when considering the findings of Granon et al. (2000) of impairments in rats following intra-PFC infusion of the DA D1 receptor antagonist SCH-23390 in an attentional task. In the Granon et al. experiments, the deficits in accuracy of performance were only apparent in rats with high levels of baseline performance, a relevant factor, in view of several other recent findings.

This complication for the hypothesis of a simple enabling role for PFC DA in working memory comes from the finding that increments in DA function can lead to decrements in working memory performance. This evidence derives from a variety of converging sources, mainly from a large group of investigators at Yale University. For example, it has been shown that elevated PFC DA turnover resulting from exposure to environmental or pharmacological stressors can disrupt working memory performance in rats in the delayed alternation paradigm, effects that can be remediated by treatment with D1 receptor antagonists (Murphy et al., 1996). Moreover, intra-PFC infusion of certain doses of the full DA D1 receptor agonist SKF-81597, can also impair delayed alternation performance through the induction of perseverative responding, an effect which can also be blocked by a D1 receptor antagonist (Zahrt et al., 1997). Finally, it has been reported that performance of a group of normal rats in this task is inversely related to DOPAC/DA indices of DA utilization or turnover within the cortex, but not the nucleus accumbens or dorsal striatum (Sahakian et al., 1985). Thus, variations in DA turnover produced by stress in the normal population hypothetically modulate working memory performance. These findings have been related to a hypothetical inverted U-shaped function relating performance to level of D1 receptor stimulation and the concomitant modulation of pyramidal cell functioning within the PFC (Arnsten, 1997; Zahrt et al., 1997). This 'Yerkes-Dodson' type hypothesis (Fig. 6) might predict improved mnemonic performance under certain conditions, especially as it appears that attentional function can be enhanced in relatively low performing rats (Granon et al., 2000). This important prediction was confirmed in recent experiments employing prelimbic infusions of the selective D1 receptor agonist SKF-81597 immediately prior to the retention test in the 'optimal foraging' paradigm described above (Floresco and Phillips, 2001). Moreover, the degree of memory enhancement was shown to be related to the initial strength of the memory trace (see Fig. 5c,d). When tested after a relatively long delay (12 h) performance was improved, whereas it was impaired after the much shorter delay of 30 min. Therefore, the inverted U shaped function relating working memory performance to level of D1 receptor stimulation in the PFC has been confirmed in normal rats.

Similar results have been forthcoming from primate studies. Thus, D1 receptor antagonists administered iontophoretically to the PFC enhance, rather than impair, stimulus processing by single units in delayed saccade paradigms (Williams and Goldman-Rakic,

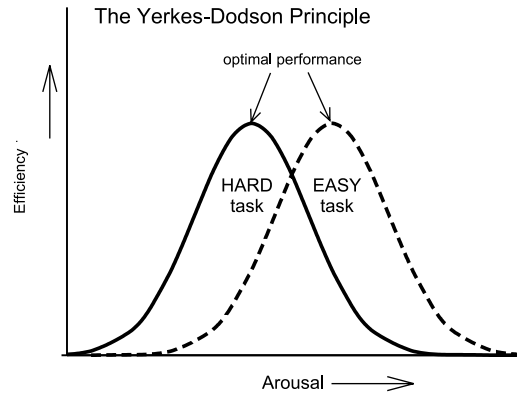

Fig. 6. The Yerkes-Dodson principle based on the inverted U-shaped function describing the relationship between arousal and efficiency. A similar relationship holds for the relationship between DA activity (e.g. within the mesocortical DA system) and performance efficiency. Note that the optimal level of performance may also be a function of the nature of the task; in the classical formulation, easy tasks being best performed at higher levels of arousal than hard or difficult tasks.

1995). The apparent discrepancy with the work of Sawaguchi and Goldman-Rakic (1990) arose from the use of larger doses in that study. Williams and Goldman-Rakic (1995) conclude that, under certain conditions, blockade of D1 receptors can potentially enhance spatial working memory performance. Overall, as for the experiments in rats, the effects of DA manipulations depend on the underlying state of the animal and its baseline level of performance, rather than simply the dose of agent administered. There is thus the potential for DA D1 receptor agonists and antagonists alike to exert opposite effects on performance, i.e. facilitation as well as impairment, depending on such conditions.

An important corollary of the Yerkes-Dodson principle as applied to PFC DA is that effects will be task-dependent – that is to say, the optimal level of performance of PFC DA may vary according to the nature of the task (see Fig. 6). We have already reviewed some evidence from the animal literature for that view. For example, whereas mesofrontal DA loss produces impairments in spatial delayed response, it is also associated with an enhancement of attentional set-shifting performance (Roberts et al., 1994). Additionally, such DA loss has no effect on the actual sequencing of spatial responses in a working memory paradigm in a task on which frontal lesions profoundly disrupt performance by inducing perseverative responding (Collins et al., 1998). Thus fluctuations in the mesofrontal DA activity, possibly representing a central correlate of enhanced stress, activation or Pavlovian arousal, impact upon behavior in ways that depend on environmental demands and the nature of the task at hand. In addition, the level of activity of the mesostriatal and mesofrontal DA systems quite often appear to be inversely related, at least in functional terms, and this may reflect in part on mutually regulatory actions (e.g. Roberts et al., 1994).

These considerations are especially important when considering complex behavior or higher cognitive functioning, in which a variety of different capacities have to be co-ordinated effectively, as originally envisaged in the Baddeley (1986) 'working memory' model. The effective planning of goal-directed behavior requires selection among several goals, the capacity to compute the optimal route to the goal (requiring attention and working memory) and the selection and the execution of the appropriate response

sequence leading to that goal (as well as the suppression of irrelevant options). Each of these processes may best be performed in different forebrain regions under different optimal levels of DA-ergic modulation. Thus, pharmacological modification of DA is likely to affect performance in different ways. Even a relatively simple procedure such as the spatial delayed response test is affected by the demands of attention and response inhibition, as well as 'holding stimuli on-line'. Consequently, it is unsurprising that other components of performance can potentially be affected by PFC DA loss, and for example, the attentional lability of the animal with prefrontal DA loss described above, while generally deleterious to good performance, may on occasions facilitate responding that requires attentional disengagement, such as the extra-dimensional shifting test (or the related, clinically-used Wisconsin Card Sort Test).

5. DA AND COGNITION IN HUMANS

Analysis of the role of DA in human cognition has been dominated by the history of the extensive research in experimental animals of the functions of cortical DA in working memory, although there are now signs of more broadly-based analyses (see also Nieoullon, 2002). The critical evidence derives from two main sources: studies of patients with disorders implicating the DA system and studies on the effects of drugs affecting DA systems in normal subjects. Such work is increasingly augmented by the use of functional neuroimaging, generally employing positron emission tomography (PET), and most recently fMRI and 'pharmacological MRI', to measure interactions between task and drug effects on regional cerebral blood flow or metabolism.

5.1. DA AND COGNITION IN CLINICAL DISORDERS

Restorations of underactive (or alternatively, reductions in overactive) DA transmission are generally assumed to be beneficial for cognitive function, motivating attempts to treat diverse disorders such as Parkinson's disease, schizophrenia, ADHD and most recently, acute brain injury.

5.1.1. Parkinson's disease

A cognitive deficit syndrome is present in idiopathic Parkinson's disease (PD), even early in its course (Taylor et al., 1986; Owen et al., 1992) as well as following MPTP-induced parkinsonism (Stern and Langston, 1985). Many of the cognitive deficits are similar to those seen after PFC dysfunction, including impairments in working memory, planning and set-shifting (Robbins et al., 1998b), especially at the early stages of the disease, although a range of other memory and learning impairments also become evident as the disease progresses (e.g. Knowlton et al., 1996). However, it is more difficult to be sure which, if any, of these deficits are linked specifically to the loss of central DA function, because of the multivariate nature of the neurochemical pathology of this neurodegenerative disease.

The cognitive deficits seen in PD patients medicated with mild clinical disability can be less than those seen in PD patients earlier in the course of the disease who are yet to receive medication (Downes et al., 1989; Owen et al., 1995). Inferences can also be made on the basis of longitudinal studies. In one large-scale study, Growdon et al. (1998) reported that levodopa improves motor function without impairing cognition in mild, nondemented PD

patients; indeed performance in tests of executive function, supposed to be sensitive to frontal lobe dysfunction, showed some benefit of medication. However, the most informative evidence comes from studies in which PD patients have their medication removed in a controlled manner. In one study of this type, Lange et al. (1992) showed that L-Dopa withdrawal from a small group ($n = 10$) of PD patients selectively impaired their performance in tests from the CANTAB battery of spatial working memory, planning and varieties of visual discrimination learning. However, it was not possible to assess performance in this relatively severely affected group of patients on tests of extra-dimensional set-shifting because of the low number of patients attempting this task. The latency, as well as the accuracy of thinking on the planning task were both affected in this group, seemingly paralleling the beneficial effects of medication on bradykinesia in PD. It is important to note that L-Dopa withdrawal did not exacerbate deficits in the patients of visual recognition memory and associative learning, showing that medication withdrawal was not producing its cognitive effects through some generalized action, for example, on fatigue or arousal.

A DA-ergic medication does not always have beneficial effects on cognition in PD. For example, there is evidence of psychosis-inducing effects of DA-ergic medication including hallucinations (Verhoeven and Tuinier, 1993), presumably related to the extensive older literature on psychotic effects of amphetamine and related drugs. Moreover, Gotham et al. (1988) provided evidence that certain aspects of cognitive performance in PD could actually be worsened by L-Dopa. They proposed a hypothesis that related the effects of L-Dopa to the pattern and course of DA loss within the striatum in PD. Those regions suffering extensive DA depletion, such as the putamen, would have their functions optimally titrated by DA medication. By contrast, those regions relatively spared in the early stages, such as the caudate and ventral striatum, would potentially be disrupted by medication, as the level of DA function would presumably be influenced supra-optimally by the drug. This hypothesis thus invokes the same Yerkes-Dodson principle as above to explain the disruptive effects of excessive PFC DA activity. Deleterious as well as beneficial effects of L-Dopa treatment have also been reported in a subset of PD patients in which the motor response to therapy is showing signs of 'wearing-off' (Kulisevsky et al., 1996). Further evidence to support the Gotham et al. (1988) hypothesis comes from a study by Swainson et al. (2000) which showed mildly medicated PD patients to perform poorly in tests of probability reversal learning – probably associated with ventral striatal and orbitofrontal function (Cools et al., 2002b) – whilst the same PD patients relatively improved on tests of spatial memory function. These findings have recently been confirmed in a detailed study on the effects of L-Dopa withdrawal, using parallel, matched groups of PD patients (Cools et al., 2001). This study compared effects of L-Dopa withdrawal in three tests of cognitive flexibility: task-set switching, attentional set-shifting (the CANTAB ID/ED test) and probability reversal. The drug selectively improved task-set switching, although it had no effect on extra-dimensional performance on the ID/ED task. These findings are consistent with the results obtained by Collins et al. (2000) following caudate DA depletion in monkeys. However, the most important findings were that L-Dopa withdrawal, consistent with the findings of Swainson et al. (2000) above, actually resulted in improved probability reversal performance, a test associated with ventral striatal-orbitofrontal circuitry on the basis of both monkey (Dias et al., 1996) and human neuroimaging (Cools et al., 2002b) findings. Cools et al. (2001) interpret these findings in terms of the pattern of DA depletion in fronto-striatal circuits (see Fig. 7). Specifically, DA loss is greater in the more dorsal, caudate-PFC than in the more ventral striatal

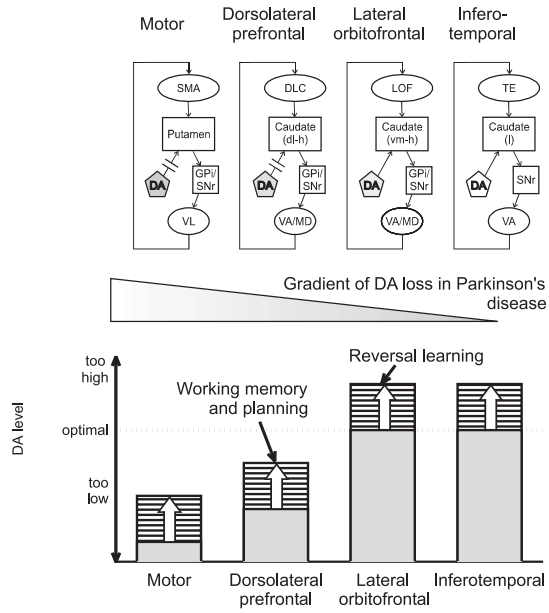

Fig. 7. Depiction of the hypothetical relationship between the locus of DA depletion in cortico-striatal 'loops', cognitive and motor performance and the effects of L-Dopa (see Swainson et al., 2000; Cools et al., 2001). Whereas some forms of cognitive performance are improved by L-Dopa in Parkinson's disease, reversal learning is impaired- possibly as a consequence of the ventral cortico-striatal loops being less severely depleted than the more dorsal 'loops', thus leading to effective 'over-dosing' of this circuitry by L-Dopa. Adapted from Swainson et al. (2000).

'loops'. Consequently, 'overdosing' of these ventral loops via systemically administered L-Dopa is more likely, according to the Yerkes-Dodson inverted U shaped function. In an independent study, Charbonneau et al. (1996) provide additional, independent evidence for this view by demonstrating that medicated PD patients were impaired in stimulus-reward, but not stimulus-stimulus learning. They hypothesized that the precise timing of DA release necessary for learning would be disrupted in PD by the disease itself, despite, or possibly because of, the medication.

There is still considerable doubt as to the loci of L-Dopa's therapeutic action in ameliorating cognitive deficits in Parkinson's disease, the striatum and the prefrontal cortex both being plausible alternatives. Recent evidence has highlighted a particular mode of interaction of L-Dopa with the network of activated circuitry produced by cognitive tasks. Data from a study using PET to measure regional cerebral blood flow (rCBF) (Cools et al., 2002a) and fMRI to measure the BOLD response (Mattay et al., 2002) converge in showing that L-Dopa appears to reduce the differences in blood flow usually existing between the task and control conditions, effectively enabling the patient to perform at an equivalent or even superior level with a diminished PFC activation in tests of planning or working memory.

5.1.2. Acute brain injury

The use of DA-ergic forms of medication in other forms of neurological disturbance is not well developed, but case study reports and experimental findings suggest possible

applications for brain-injured patients. For example, McDowell et al. (1998) investigated the effects of a low dose of the DA D2 receptor agonist bromocriptine on working memory and other executive forms of cognitive function in individuals with traumatic brain injury in a double-blind cross-over trial with placebo. Consistent with the findings for Parkinson's disease, bromocriptine improved performance on some, but not all tasks thought to be subserved by the PFC. Also consistent with the Parkinson's disease literature, no effects were observed for control tasks not thought to be subserved by the PFC. More controversially, and seemingly at odds with both the animal literature and findings on normal individuals to be reviewed here, bromocriptine exerted no effects on those working memory tasks with minimal additional demands on executive function.

5.1.3. Schizophrenia

Making inferences about the functions of DA in cognition is problematic in the case of schizophrenia, because antipsychotic medication may well produce indirect effects on performance by the remediation of disruptive positive symptoms. Moreover, neuroleptic drugs (e.g. Williams et al., 1998) can impair cognitive functioning (King, 1990). In an extensive review, Mortimer (1997) concluded that much remained unclear about whether neuroleptic treatment affected the cognitive deficit syndrome present in schizophrenia. The effects of conventional neuroleptics are quite small, often being beneficial and related to the remission of psychosis. The possibility that the so-called atypical neuroleptics, such as clozapine exert 'cognitive facilitation', as well as 'cognitive sparing' effects, needs to be resolved using more sophisticated neuropsychological methods and study designs.

The potential complexity in this area can be gauged from a functional neuroimaging study using PET to measure rCBF in normal and unmedicated schizophrenic subjects following challenge with apomorphine or placebo (Dolan et al., 1995) – extending an analogously-motivated study of the effects of d-amphetamine in schizophrenia (Daniel et al., 1991). Dolan et al. found that rCBF was enhanced in the anterior cingulate cortex in the schizophrenic patients under the conditions of a verbal fluency task. However, one problem of interpretation for these data is knowing whether the effects of apomorphine depended on an enhancement of DA neurotransmision, or alternatively on a reduction, via its pre-synaptic action at D2 receptors. Another problem of interpretation is posed by the lack of reported data for verbal fluency performance in that study. So, although the therapeutic implications may be evident, the actual impact on cognition of cortical actions of apomorphine in the schizophrenic or normal individuals, is unclear.

5.1.4. Attention deficit/hyperactivity disorder

Similar uncertainties as to whether treatment is 'damping down' unwanted activity or boosting deficient functioning also hinder our understanding of the basis of the apparently effective strategy of treating ADHD with methylphenidate and other amphetamine-like compounds (Solanto et al., 2000; Mehta et al., 2000a). Converging evidence implicates the DA-ergic system and the prefrontal and nigrostriatal regions in the pathophysiology of childhood ADHD and prefrontal DA-ergic dysfunction in adult ADHD (Ernst et al., 1998), but it remains unclear to what extent the beneficial effects of drugs, such as methylphenidate (Ritalin) depend on modulation of DA-ergic or noradrenergic neurotransmission, or both. The neural site of such effects is also unclear. Vaidya et al. (1998) have employed fMRI in a 'Go/No Go' functional imaging paradigm to show that

methylphenidate attenuated blood flow in the basal ganglia of normal children, but increased blood flow in children with ADHD. On the other hand, equivalent degrees of frontal activation were seen in both groups. Improvements in behavioral performance were also seen in both groups following the drug, but, as in the case of Parkinson's disease with L-Dopa, it is difficult to be sure at which neural loci the stimulant is acting to produce these effects. Studies by Mattay et al. (1996) and Mehta et al. (2000b) on the effects of d-amphetamine and methylphenidate, respectively, in normal volunteers, implicate cortical networks that include the dorsolateral PFC. These latter experiments also utilized tasks that normally require PFC functioning (performance on the WCST and self-ordered spatial working memory tasks, respectively). Thus, the identity of the neural networks upon which stimulant drugs exert their effects on performance – for both normal and clinical populations – may hinge on the nature of the task under study.

5.2. EFFECTS OF DA-ERGIC DRUGS ON COGNITION IN NORMAL HUMAN VOLUNTEERS

Early research showing that amphetamine-like drugs had beneficial effects on vigilance functions has generally held up (Koelega, 1993). Despite its use in ADHD, the effects of methylphenidate on other aspects of cognition until recently have not been widely investigated. Clark et al. (1986) showed that methylphenidate (0.65 mg/kg p.o.) reversed impairments in a dichotic auditory attention task produced by the neuroleptic drug droperidol. By itself, however, methylphenidate had little effect except to enhance subjective increases in elation, energy and alertness. It was not possible to attribute significant improvements of a similar oral dose in CANTAB tests of self-ordered spatial working memory and planning function (Elliott et al., 1997), which were limited mainly to the first test session. Indeed, when taken in a second session, the drug sometimes increased the speed of responding on certain tests, such as some forms of the Tower of London planning task, at the expense of reduced accuracy. Also evident were effects of enhanced memory retrieval, consistent with other data (Evans et al., 1986). Another study (Rogers et al., 1999) has shown that methylphenidate (at the same dose to that employed by Elliott et al.,), can improve performance on an extra-dimensional set shift task, similar to that employed in monkeys by Roberts et al. (1994), but at the cost of slowing performance and increasing errors in the control test of intra-dimensional set shifting. These results are important in showing that it is possible to demonstrate improvements in normal individuals treated with methylphenidate, as well as in patients with ADHD. However, consistent with the animal and clinical data reviewed above, other functions may also show impairment. Thus, drugs such as methylphenidate (and presumably other psychomotor stimulants) appear to place the subject into an altered mode of functioning that is optimal for certain forms of performance, such as working memory, memory retrieval functions and responding to previously irrelevant stimulus dimensions, though at the cost of other capacities. The challenge now is to determine the contribution of DA itself to these effects and also to identify the neural loci of the drug-task interactions in the intact brain.

The most direct means of addressing this challenge is to study the effects of specific DA-ergic agonists and antagonists on human cognition, incorporating a functional imaging approach wherever feasible. Unfortunately, the lack of sufficiently selective compounds suitable for administering to normal human volunteers (e.g. without emetic and dyskinetic side-effects) has retarded progress. DA D2 receptor antagonists generally impair cognitive function in normal volunteers. However, the impairments are not simply linked to sedative

actions: sulpiride produces relatively little effect on tests of sustained attention and associative learning that are sensitive to benzodiazepines such as diazepam (Mehta et al., 1999). In that study, however, sulpiride (400 mg p.o.) did produce a pattern of impairments qualitatively similar to that seen in Parkinson's disease, including deficits in spatial but not visual pattern recognition memory, planning performance and attentional set-shifting – again reflecting capacities mediated by fronto-striatal systems.

The greater predominance of D2 receptor binding in the striatal rather than the cortical regions implicates the striatum as a likely site of action of many of these effects. This is consistent with evidence of correlation between DA D2 receptor binding in both normal volunteers and patients. For example, Volkow et al. (1998) found several significant correlations between performance measures (for tasks administered outside the scanner) and indices of D2 receptor binding using [11C]-raclopride. Although these correlations were greatest for motor tasks such as finger tapping, significant correlations were also found for measures of cognitive function, including performance on Raven's Matrices, and the Stroop and WCST tests (categories attained measure), even after correcting for the considerable decline in D2 receptor binding that occurs with normal aging. Lawrence et al. (1998) also found that several aspects of performance on spatial working memory and planning tasks exhibited significant correlations with indices of striatal D2 receptor binding in patients at various stages of Huntington's disease. An exciting prospect would be to attempt to confirm such findings using functional imaging paradigms to produce DA receptor displacement – in other words, directly to relate DA release to cognitive performance in conscious human subjects. Some progress in attaining this goal has been made in a seminal study by Koepp et al. (1998). They showed that performing a motivating video-game reduced binding of raclopride to DA receptors in the region of the ventral striatum, presumably because of striatal DA release engendered by the task. Whilst the nature of the cognitive operations engaged by this task within the striatum cannot be specified by this study alone, it nevertheless offers great promise for future advances if used in combination with the other approaches surveyed here.

It has only proven feasible to assess performance altering effects of DA D2 receptor agents such as bromocriptine, or alternatively, of mixed D1–D2 agents such as apomorphine and pergolide. Even though studies so far have been limited, significant improvements in some aspects of cognitive performance have been seen in most of these. The main exception used a rather different cognitive task: Grasby et al. (1992) showed that apomorphine (5 and 10 μg s.c.) impaired learning of an auditory-verbal word list in a PET-scanning paradigm were related to its effects to reduce prefrontal cortical regional cerebral blood flow.

Improvements in cognitive function have been mainly observed in visuospatial working memory tasks. Luciana et al. (1992) demonstrated that bromocriptine (2.5 mg p.o.) enhanced the accuracy of performance in a delayed saccade task. Luciana et al. (1998) extended the result to show improvement of memory for spatial, but not object, cues at a lower dose of bromocriptine (1.25 mg), and they further demonstrated pharmacological specificity by demonstrating opposed effects of a serotoninergic drug (fenfluramine) (Luciana and Collins, 1998). By contrast, Muller et al. (1998), using a different delayed matching working memory task in which subjects had to match the location of a complex visual pattern within a spatial frame of reference, failed to find significant improvement with bromocriptine (2.5 mg). They were able, however, to demonstrate significant benefits of the mixed DA agonist, pergolide, which they attributed potentially to its D1 receptor agonist properties. Further illumination on the controlling variables for these effects has

come from findings of Kimberg et al. (1997) that the effects of bromocriptine (2.5 mg) in normal young adults depended on their baseline working memory capacity. High capacity subjects performed more poorly on a range of executive and working memory tasks whereas low capacity subjects performed better after this dose. This is reminiscent of the inverted U-shaped Yerkes-Dodson-like functions already shown above to be important for determining the effects of DA-ergic manipulations, although Kimberg et al. (1997) invoke more computationally rigorous applications of the sigmoid activation function (Servan-Schreiber et al., 1990). Kimberg et al. thus failed to replicate Luciana et al. (1992) effects with a task that was slightly different from that used by them, in its inclusion of a central distractor condition. While Kimberg et al. suggest that the discrepancy between their results and those of Luciana et al. might reflect differences in the baseline working memory capacities of their subject samples, another plausible explanation is that the less complex visuospatial form of the memory task, requiring memory for only the location of a simple stimulus at a single spatial location, may be more sensitive to improvement than the more complex forms of this task. In support of this, Mehta et al. (2001) have shown that a lower dose of bromocriptine (1.25 mg) improves performance of the CANTAB spatial span task but not its self-ordered spatial working memory equivalent. Of relevance to the earlier studies of effects of L-Dopa on Parkinson's disease, normal volunteers performed the probability reversal task more poorly under the effects of bromocriptine, at the same dose that significantly improved visuospatial memory. This is of course, is analogous to the differential effects of L-Dopa on similar tasks (Cools et al., 2001), suggesting that the effects of DA agents in these tasks may have some fundamental dose-response differentials, even in subjects for which their controlling fronto-striatal systems are intact.

The effects of DA-ergic agents, such as bromocriptine, are thus both weak and subtle, depending on both the nature of the task under study as well as on baseline capacities of normal individuals. This might be because a direct agonist is a less effective way of enhancing normal function, as compared to a drug that modulates neurotransmitter release. While the enhancements in cognitive function are theoretically important, it seems likely from the data obtained so far that enhancement is only likely to be achieved in certain situations and only at the possible cost of inefficiency in other domains. The apparent susceptibility of individuals low in baseline working memory capacity to cognition-enhancing effects of bromocriptine may be a useful indicator for the use of D2 receptor agonists in clinical applications. The related issue of individual differences is how these may arise from genetic variability.

6. CURRENT FOCI AND FUTURE DIRECTIONS

Study of the roles of the central DA systems has undergone a significant shift in the last decade or so, as their evident importance for motor and motivational processes has been perhaps more than matched by a realization of their growing significance for understanding cognitive, and especially prefrontal cortical, function. At the same time, it should be emphasized that it is also becoming obvious that segregating motor, motivational and cognitive functions is an artificial enterprise: the control of action certainly has cognitive components, and decision-making processes are increasingly informed by models of reinforcement learning in which DA plays an important role (e.g. Montague et al., 1996). This discussion considers future directions which, hopefully, will

lead to an integrated understanding of the functional role of DA in cognition that takes into account behavioral, anatomical and electrophysiological data within a framework embracing new directions such as computational modeling, pharmacological fMRI and functional genomics.

6.1. CELLULAR ACCOUNTS OF PREFRONTAL DA FUNCTION: ELECTROPHYSIOLOGY AND COMPUTATIONAL MODELING

While it has not been feasible to provide a full review of computational modeling (but see chapter by Arbuthnott and Wickens), this is becoming an increasingly important tool at many different levels of analysis of the functions of DA systems. Such modeling however, is based on quite divergent standpoints which include, at one end of the range, abstract, parallel distributed processing, neural network frameworks (Servan-Schrieber et al., 1990; Braver et al., 1999), and at the other end, modeling based more particularly on effects of DA on neurons themselves (e.g. Hodgkin-Huxley equations for the operation of ion channels), and on other electrophysiological data (Durstewitz et al., 2000; Dreher and Burnod, 2002; Dreher et al., 2002). The modulation of neural networks similar to those presumably operating within regions such as the prefrontal cortex and striatum by 'slow' chemical transmitters, such as DA is in fact seldom taken into account in abstract computational modeling. Such influences are generally modeled by assuming they act on an entire group of neurons (via diffusion and volume transmission) and change more slowly than the variables modeling the dynamics of the network. Thus, for example, the Cohen group (e.g. Servan-Schrieber et al., 1990) postulate that DA increases signal-to-noise ratios for neurons as a gain parameter of a sigmoid function; this would have the effect of enhancing both excitatory and inhibitory inputs in a relatively time-independent manner. However, other modes of action of prefrontal DA also have to be taken into account: its phasic, fast signaling effects in the context of reinforcement and 'error feedback' (Schultz et al., 1992); its tonic, relatively delayed post-synaptic effects (Moore et al., 1999); and current evidence indicating that DA has a general inhibitory effect on PFC neuronal activity (see Dreher and Burnod, 2002). Recent simulations of the influence of DA on intrinsic and synaptic currents of PFC neurons have supported the concept of DA's involvement in working memory, specifically by reducing the impact of retrograde interference from stimuli intervening in the delay. This action would thus enhance the robustness and stability of representations of such stimuli as the location of a goal (Dustrewitz et al., 2000). We have argued earlier that this stabilization of representations need not depend entirely on the holding of information on-line, but may also serve to prevent attentional lability and resultant distractibility (Crofts et al., 2001).

The recent theory of Dreher and Burnod (2002) points out that it may be more biologically plausible to assume that the actions of DA are delay-dependent. For example, whereas Braver et al. (1999) postulate that the actions of DA in the PFC reflect a gating mechanism by which incoming inputs are facilitated at the time of their presentation, the Dreher and Burnod hypothesis is that following firing of DA neurons, the neurotransmitter acts in a phasic manner at post-synaptic D1 DA receptors for a few seconds to restrict excitatory inputs arriving at superficial layers of the PFC, thus protecting pyramidal cells in the deep layers from noisy inputs. This is consistent with neurophysiological findings (e.g. Yang and Seamans, 1996). Dreher and Burnod (2002) have extended this model to include a functional role for the tonic mode of DA release that extends the post-stimulus inhibitory actions from seconds to minutes and even hours. It is

particularly significant that these methods for analyzing the functional role of DA increasingly take up the challenge of simulating and also predicting results of behavioral experiments, for example, of delayed response tasks (Dreher and Burnod, 2002). We can certainly expect more advances in this area in the next few years to enable us to explain, and hopefully to predict, the precise nature of the effects of neuromodulation by DA on tasks sensitive to prefrontal dysfunction in animals and humans. This may be important, for example, for explaining why performance of some tasks may exhibit facilitation following DA agents, whereas others may be affected in the opposite fashion. This type of theorizing however is not able to account much for the apparent individual differences in response to DA agents in tests of cognition (e.g. Kimberg et al., 1997; Granon et al., 2000); to explain these, a genetic perspective may prove to be useful.

6.2. PREFRONTAL DA AND FUNCTIONAL GENOMICS

Abnormalities in the prefrontal cortical processing, including cognitive functioning, have recently been associated with functional polymorphisms of the catechol-O-methyl transferase (COMT) gene. By modifying the enzyme's activity, these polymorphisms appear to have a special impact on prefrontal DA and can affect performance on 'fronto-executive' type tasks (Egan et al., 2001). The evidence for specific effects on prefrontal DA comes in part from experiments in animals, such as COMT knockout mice, which have increased prefrontal DA (but not noradrenaline) (Gogos et al., 1998). Moreover, pharmacological experiments with both rats and monkeys have implicated COMT in the regulation of extracellular DA in the PFC (Elsworth et al., 1987; Karoun et al., 1994). Moreover, COMT inhibitors have been reported to improve working memory in rats (Liljequist et al., 1997). The theory is that COMT assumes a much greater role in regulating DA in the PFC because of the relative paucity of DA synaptic transporters there.

The COMT gene is a plausible candidate for influencing the prefrontal DA function because it contains a single nucleotide polymorphism at position 472 (guanine-to-adenine substitution) which is a valine-to-methionine alteration, resulting in reductions in COMT activity. The polymorphism first becomes evident in phylogenetic terms in humans. Thus humans with the val/val genotype will tend hypothetically to have more rapid inactivation of released PFC DA relative to the met/met genotype, with the val/met heterozygote, intermediate between these. These changes in PFC DA function hypothetically should be associated with relatively impaired performance on tests of cognition sensitive to frontal lobe dysfunction. This prediction has been confirmed for WCST and working memory ('n-back' tasks) performance, with the COMT genotype predicting 4% of WCST performance (Egan et al., 2001; Weinberger et al., 2001; Goldberg et al., 2003 – see Fig. 8). Furthermore, the val/val individuals tend to be the ones who benefit from enhancing effects of amphetamine on performance, as might have been predicted, for example, from the effects of methylphenidate on spatial working memory performance (Mehta et al., 2000a,b), where the drug was most effective in those normal volunteers with the worst digit span scores. Finally, using the n-back test of working memory, fMRI studies of the three genotypes found that there was lesser 'physiological efficiency' (i.e. an exaggeration of the fMRI response in the PFC) in individuals with the val/val genotype, as compared with met/met individuals. These data can in principle be used to account for striking individual, generally baseline-dependent, differences in performance within the normal population. Thus, we might predict impaired performance by D1 receptor antagonists in met/met

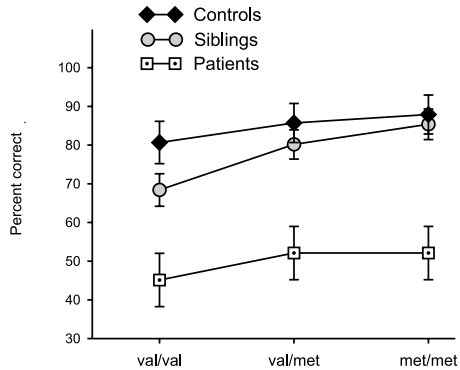

Fig. 8. Effect of COMT genotype on n-back working memory performance in normal subjects, schizophrenic patients and their siblings. Reproduced with permission from the authors and publishers. Adapted from Goldberg et al. (2003).

subjects, but quite the opposite for the val/val population. The COMT polymorphism may be relevant to understanding syndromes such as ADHD or schizophrenia (Weinberger et al., 2001). In one recent study (Goldberg et al., 2003) strong evidence for a genotype-dependent effect on a one-back working memory task was found for normal subjects, schizophrenic patients and their siblings, with the val/val subjects performing worst and the met/met subjects best, producing a significant main effect of genotype (see Fig. 8). The precise relationship, therefore, between this polymorphism and schizophrenia is presently unclear. However, future study of the COMT and other polymorphisms promises greatly to contribute to our understanding of the functions of PFC DA in humans as well as to the resolution of its involvement in schizophrenia and other forms of psychopathology, including ADHD and depression.

6.3. INTERACTIONS WITH OTHER SYSTEMS WITHIN THE PREFRONTAL CORTEX

Ultimately, the possible roles identified for DA in cognition in this chapter will have to be mapped onto the complexities of effects of DA at a cellular level, including its interactions with other neurotransmitters, for example, within the PFC. Thus, it is likely that the D1, D2 and D4 receptors have distinct functional roles there, although these have yet to be established at a functional level (c.f. the possible involvement of D4 receptors in ADHD, see Solanto et al., 2001). It is also becoming clearer about the ways in which prefrontal DA regulates the outflow of information encoded in the activity of the prefrontal cortical pyramidal cells, through its interactions with prefrontal GABA (Chesselet, 2002) and glutamate (Konradi et al., 2002) neurons. Of additional significance is the nature of the mesocortical DA-ergic modulation of prefrontal function in relation to the contributions of the noradrenergic, serotoninergic and cholinergic ascending systems. For example, one possible scheme postulates that serotoninergic activity affects D1-dependent DA-ergic modulation, which in turn directly regulates the impact of acetylcholine release on prefrontal functioning (Acquas and Di Chiara, 2002). Clearly, these mechanisms will have to be worked out in considerably greater detail. However, the questions also arise as to what extent these systems may function relatively independent of one another, and under precisely what environmental (internal or external) circumstances do fluctuations in these

systems occur? The ascending systems are variously implicated in such processes as reinforcement, stress, arousal and mood, but currently very little analysis is being applied to the distinctive, as well as the common, elements of these processes. At a theoretical level, this will allow, for example, more accurate computational modeling of the ways in which different functional states optimize and constrain the efficiency of executive and cognitive operations.

7. ACKNOWLEDGMENTS

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CHAPTER VIII

Functional neuroanatomy of hypothalamic dopaminergic neuroendocrine systems

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ABSTRACT

Diencephalic DA neuroendocrine systems comprise several anatomically and functionally distinct groups of neurons including tuberoinfundibular, incertohypothalamic, periventricular-hypophysial, periventricular and ventrolateral DA neurons. This chapter provides an overview of the role of these diencephalic DA neurons in the regulation of pituitary hormone secretion, with special emphasis on their functional neuroanatomy, hormonal feedback regulation, and regulatory neuronal pathways which mediate changes in hormone secretion during various physiological states. These aspects are considered with regard to gender specific changes in neuronal function that occur throughout the life span of animals from ontogeny and differentiation through puberty, adulthood and aging. Included is information gained from a variety of classical and modern molecular approaches to the study of the regulation and function of these neurons in rats, mice and photoperiod-sensitive seasonal breeding species, such as sheep and cattle. Where appropriate, the advantages and limitations of the technical aspects of these approaches as applied to the study of diencephalic DA neuronal systems are discussed.

1. INTRODUCTION

The mammalian brain contains several anatomically distinct dopamine (DA) neuronal systems that differ in their neurochemical characteristics, regulatory afferent and efferent neuronal connections and physiological functions. Of these, the best studied are the mesotelencephalic systems comprising nigrostriatal DA neurons which regulate motor activity and mesolimbic DA neurons which mediate emotionally driven affective behaviors (as reviewed elsewhere in this volume). Differences in properties of these DA neuronal systems are currently being exploited in the development of selective therapeutic strategies for the treatment of Parkinson's disease and schizophrenia. It is, however, misleading to consider mesotelencephalic neurons as characteristic of all DA neurons in the brain because marked differences in the regulation and function of other DA neurons have been noted, especially those in the diencephalon. Indeed, in the rat brain, the numbers of DA neurons in the hypothalamus and adjoining subthalamus rival those of midbrain mesotelencephalic DA neurons, yet there are considerably fewer studies on the regulation of these DA neurons. Although there are minor species differences in the

abundance and distribution of diencephalic DA neurons, these neurons play important roles throughout mammalian phylogeny in the neuroendocrine regulation of pituitary hormone secretion.

There have been several recent reviews on the neuroendocrine function of hypothalamic DA neuronal systems, but these have focused mainly on tuberoinfundibular (TI) DA neurons and the regulation of pituitary prolactin secretion (Pan, 1996; Freeman et al., 2000; Ben-Jonathan and Hnasko, 2001). The overall purpose of this chapter is to provide an overview of the functional role of these and other diencephalic DA neurons in the regulation of pituitary hormone. Emphasis is placed on their functional neuroanatomy, hormonal feedback regulation, and regulatory neuronal pathways mediating changes in hormone secretion during various physiological states. These aspects are considered with regard to gender specific changes in neuronal function that occur throughout the life span of animals from ontogeny and differentiation through puberty, adulthood and aging. This chapter incorporates new information gained from a variety of classical as well as modern molecular approaches to the study of the regulation and function of these neurons in rats, mice and (in some cases) photoperiod-sensitive seasonal breeding species such as sheep and cattle. Where appropriate, the advantages and limitations of the technical aspects of these approaches as applied to the study of diencephalic DA neuronal systems will be discussed.

2. ANATOMY OF DIENCEPHALIC DA NEURONAL SYSTEMS

Details of the anatomy of DA neuronal systems in the rat diencephalon have been described by Björklund and Lindvall (1984) and the location of their perikarya are depicted schematically in Fig. 1 using the alphanumeric system of Dahlström and Fuxe (1964). The numbers of DA perikarya in the rat diencephalon (A_{11}, A_{12}, A_{13} and A_{14}/A_{15}; van den Pol et al., 1984) are comparable to those in the substantia nigra (A_8 and A_9; German et al., 1983; German and Manaye, 1993) and ventral tegmental area (A_{10}; German et al., 1983; German and Manaye, 1993), which are generally considered to be the major loci of DA neurons in the brain. The most familiar diencephalic DA neurons are those that comprise the TIDA system; perikarya of these neurons (A_{12}), which are located in the mediobasal hypothalamic arcuate nucleus (ARC) and adjacent periventricular nucleus, project to the external layer of the median eminence. Although TIDA neurons have been studied more extensively than other DA neurons in the diencephalon they actually represent a minority of these DA neurons (Reymond et al., 1984). A majority of diencephalic DA neurons are located in dorsal regions of the hypothalamus and ventral thalamus, and the regions adjacent to the third ventricle. A small number of relatively large DA perikarya (A_{11}) are located in the posterior regions of the dorsal hypothalamus and the periventricular gray of the central thalamus; axons from these neurons project to the spinal cord (Skagerberg and Lindvall, 1985). The presence of DA receptors in the superficial layers of the dorsal horn and in the pars centralis of the spinal cord suggest a role for these neurons in sensory and nociceptive processing, as well as sensorimotor integration (Van Dijken et al., 1996; Levant and McCarlson, 2001). Due to the lack of evidence of a neuroendocrine function of these diencephalospinal DA neurons, they will not be discussed further in this chapter.

The DA perikarya of incertohypothalamic (IH) DA neurons (identified as the A_{13} cell group) are clustered in the rostral portion of the medial zona incerta (MZI). These densely

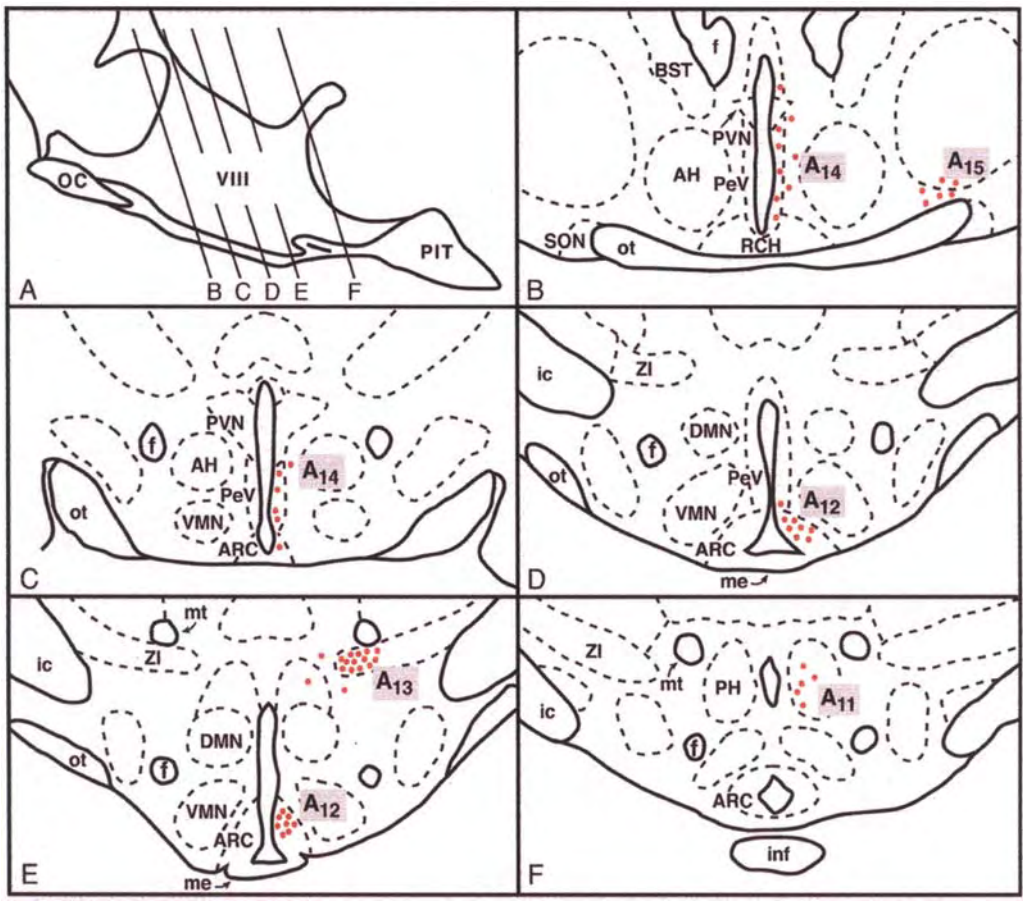

Fig. 1. Location of dopaminergic perikarya (A_{11}–A_{15}) are depicted schematically on frontal sections (B–F) through the diencephalon of the rat. Section A is a sagittal view of the rat brain depicting the rostrocaudal location of frontal sections B–F. Abbreviations: AH, anterior hypothalamus; ARC, arcuate nucleus; BST, bed nucleus of the stria terminalis; f, fornix; ic, internal capsule; inf, infundibulum; me, median eminence; mt, mamillothalamic tract; OC, optic chiasm; ot, optic tract; PH, posterior hypothalamus; PIT, pituitary gland; PeV, periventricular nucleus; PVN, paraventricular nucleus; RCH, retrochiasmatic area; SON, supraoptic nucleus; VMN, ventromedial nucleus; ZI, zona incerta.

packed DA neurons have extensive dendritic processes oriented in the ventral plane which extend into the dorsomedial nucleus of the hypothalamus (Chan-Palay et al., 1984; van den Pol et al., 1984). Early reports suggested that efferents of IHDA neurons project locally into the surrounding regions of the hypothalamus (Björklund and Lindvall, 1984), but more recent studies reveal that IHDA neurons project to the central nucleus of the amygdala, horizontal diagonal band of Broca and hypothalamic paraventricular nucleus (Eaton et al., 1994; Wagner et al., 1995).

DA neurons projecting to the neural and intermediate lobes of the posterior pituitary were reported initially to originate from rostral A_{12} cells in the ARC and referred to as tuberohypophysial DA neurons (Björklund et al., 1973). More recent studies (Kawano and Daikoku, 1987; Goudreau et al., 1992, 1995) revealed that DA neurons projecting to the intermediate lobe of the posterior pituitary originate from a subpopulation of A_{14} DA

cells in the periventricular nucleus. In this review DA neurons projecting to the intermediate lobe of the pituitary will be identified as the periventricular-hypophysial (PH) DA neurons, although in the majority of earlier references these neurons are referred to as tuberohypophysial DA neurons. The remaining A_{14} periventricular (PeV) DA neurons are believed to project laterally into adjacent regions (e.g. medial preoptic area, anterior hypothalamic area). A_{15} DA neurons show remarkable species differences in their prevalence, distribution, neurochemical nature and function (Tillet and Thibault, 1989; Tillet et al., 1990; Van Vulpen et al., 1999). These neurons are prominent in the ventrolateral hypothalamus of seasonal breeding species such as sheep (Tillet and Thibault, 1989), and are believed to mediate steroid hormone suppression of gonadotropin secretion during anestrus in ewes (Gayrard et al., 1994; Lehman et al., 1996). Additional details of the distribution of TIDA, IHDA, PHDA, PeVDA and ventrolateral A_{15} DA neurons are provided in the following sections dealing with each of these neuronal systems.

2.1. ONTOGENY OF DIENCEPHALIC DA NEURONS

The ontogeny and differentiation of diencephalic DA neurons progresses through four chronological stages consisting of: (1) formation of neurons from neuroepithelial precursor cells, (2) expression of biosynthetic enzymes, synthesis of DA and its precursors, and development of mechanisms for DA release and reuptake, (3) formation of efferent projections, and (4) formation of afferent connections and synaptogenesis (for review see Ugrumov, 2000). In the rat, neuroepithelial progenitor cells destined to become diencephalic DA neurons originate in the anterior neural ridge of the somite embryo, a site distinct from the origin of precursors of mesotelencephalic DA neurons (Hymes and Rosenthal, 1999). By the 13th day of embryonic life these cells begin to express mRNA for tyrosine hydroxylase (TH), the rate limiting enzyme in DA synthesis (Coulon et al., 1990), and at this stage sparse TH-immunoreactive (IR) cells can be found in the septal region and scattered throughout the lateral hypothalamus (Daikoku et al., 1986; Ugrumov et al., 1989a). During late gestation, these cells migrate into clusters within identifiable hypothalamic nuclei and differentiate into neurons with distinct bipolar or multipolar processes (Ugrumov et al., 1989a). At birth, these neurons are capable of synthesizing, releasing and recapturing DA, albeit to a lesser extent than in neonates or adults (Borisova et al., 1991).

Differentiation continues throughout the early postnatal period such that by postnatal day 9 three distinct populations of TH-IR neurons can be identified in the diencephalon, each located in discrete regions corresponding with the A_{12}, A_{13} and A_{14} groups in adult animals (Dahlström and Fuxe, 1964; van den Pol et al., 1984). The first group consists of small unipolar and bipolar neurons with short, narrow unbranched processes that occupy the ARC and are regularly arranged along the ventral surface of the hypothalamus. The second population of TH-IR neurons is located in the zona incerta and is composed largely of multipolar cells with long highly ramified processes. The third population includes large bipolar neurons with long crooked processes that are mainly found in the periventricular nucleus (Ugrumov et al., 1989a). Thus, prior to the onset of puberty the distribution patterns and morphological features of diencephalic DA neurons are similar to those in adult animals (van den Pol et al., 1984; Borisova et al., 1991). Changes in the activity and function of diencephalic DA neurons associated with puberty will be discussed later in this chapter.

There are sexual differences in the ontogeny of diencephalic DA neurons that become evident early in embryological development. Dissociated cell cultures raised from the diencephalon of male and female rat fetuses from day 14 of gestation show striking differences in morphology with outgrowth of TH-IR processes initially proceeding at a faster rate in cultures of female than in male cells (Reisert et al., 1989). The uptake capacity and the evoked release of DA is also twice as high in cells derived from females. These differences disappear in cultures from gestational age 17 and are not influenced by treatment with testosterone, dihydrotestosterone or estradiol, suggesting that sexual differentiation of diencephalic DA neurons is controlled by the genotype rather than by epigenetic actions of gonadal steroids (Reisert et al., 1989). One possible explanation for these differences could be an early proliferation of female precursor cells which would give them a head start in the initiation of differentiation over male DA cell precursors. Temporal differences between the origin of female and male neurons have been described for DA precursor cells of the sexually dimorphic nucleus of the preoptic region (Gorski and Jacobson, 1982).

Sexual differences have also been observed in the development of TH-IR neurons in the mediobasal hypothalamus with respect to their number and size, location, amount of TH, and number of cells expressing 3,4-dihydroxyphenylalanine (DOPA) decarboxylase (DDC) (Balan et al., 2000). Beginning on the 20th day of gestation through postnatal day 9, the number of TH-IR neurons in males exceeds that in females, primarily due to higher numbers of neurons in the ventrolateral (VL) as opposed to the dorsomedial (DM) region of the ARC. In contrast, the size of TH-IR cells and their optical densities (i.e. amount of TH) are higher in females, especially in the DM-ARC. Females also have greater numbers of DDC-IR neurons throughout the mediobasal hypothalamus during late gestational and early postnatal development (Balan et al., 2000). These sexual differences in the distribution and relative activity of TH-IR neurons in the mediobasal hypothalamus of female and male prepubertal rats are strikingly similar to those present in gonadally intact adult animals (Cheung et al., 1997).

During the neonatal period (days 3–15) DA neurons in the ARC, MZI and substantia nigra of male and female rats show a similar pattern of development, with levels of TH mRNA increasing about 3–4-fold. TH catalytic activity in the stalk/median eminence also increases 2–3 times during this period. During the next 20–25 days these levels remain relatively constant until the peripubertal period (days 35–40) when TH mRNA levels exclusively in TIDA perikarya in the ARC (and TH catalytic activity in their terminals in the median eminence) increase approximately 3-fold in females, but not males. This sexual difference in TIDA neurons is maintained throughout adult life (Arbogast and Voogt, 1991a). It has been suggested that changes in TIDA neurons in female rats during puberty may be related to the emergence of progesterone secretion associated with the initiation of estrous cyclicity.

2.2. DISTRIBUTION OF DA NEURONS IN THE DIENCEPHALON

2.2.1. Tuberoinfundibular DA neurons (A_{12})

In adult rats, perikarya of TIDA neurons, originally described as comprising the A_{12} cell group (Dahlström and Fuxe, 1964), are distributed throughout the rostrocaudal extent of the ARC and adjacent periventricular nucleus of the mediobasal hypothalamus. Two populations of TH-containing neurons have been identified based on their neurochemical

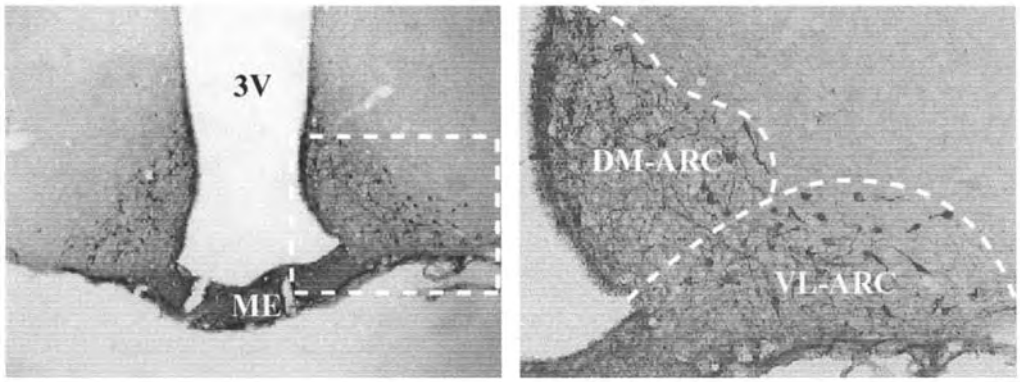

Fig. 2. Distribution of TH-IR neurons in the arcurate nucleus (ARC). (Left Panel) Low power image depicting the ARC and median eminence (ME) in relation to the third ventricle (3V). Dashed lines indicate inset shown in the right panel. (Right Panel) High power image depicting TH-IR perikarya in the dorsomedial (DM) and ventrolateral (VL) subdivisions of the ARC. TH-IR neurons in the DM-ARC and VL-ARC are separated by a TH-IR cell-free zone demarcated by a line extending laterally at an angle of 30° from the lateral aperature of the third ventricle (Meister et al., 1988).

phenotypes, and the size and location of their perikarya in the DM and VL regions of the ARC (Fig. 2; Everitt et al., 1986).

Relatively small TH-IR perikarya found in the DM-ARC have dendrites oriented in the dorsoventral plane (Chan-Palay et al., 1984; van den Pol et al., 1984), and axons that project ventrally to terminate in both medial and lateral aspects of the median eminence (Selmanoff, 1981; Björklund and Lindvall, 1984). These neurons lack true synapses, and DA released from their axon terminals into the perivascular spaces diffuses through fenestrated capillaries and is transported in the hypophysial portal blood to the anterior pituitary where it activates D_2 receptors on lactotrophs thereby inhibiting the secretion of prolactin. DA synthesis in axon terminals of TIDA neurons in the median eminence begins around postnatal day 4 and increases throughout the first three weeks when DA levels reach levels comparable to that found in adults (Smith and Simpson, 1970). In adults there are no sexual differences in the number of TH-IR neurons in the DM-ARC (Cheung et al., 1997) or the density of DA fibers (Smith and Simpson, 1970) in the median eminence. However, neuronal expression of Fos related transcription factors (Cheung et al., 1997) and TH mRNA (Arbogast and Voogt, 1990, 1991a) in the DM-ARC, and the amount of TH (Porter, 1996) and synthesis of DA (Demarest et al., 1981) in the median eminence are all greater in females as compared with males. The roles of prolactin and gonadal steroid hormones in determining these sexual differences in activity of TIDA neurons will be discussed later in this chapter.

TH-IR neurons in the VL-ARC are larger in size than those in the DM-ARC (Everitt et al. 1986), with dendrites oriented in the mediolateral plane (Chan-Palay et al., 1984; van den Pol et al., 1984) and axons that terminate in the lateral portion of the median eminence (Meister and Hökfelt, 1988). These neurons lack DDC (Everitt et al., 1986; Meister et al., 1988) and do not express DA transporter mRNA as do DA-containing TH-IR neurons in the DM-ARC (Meister and Elde, 1993). Since these 'DOPAergic' neurons do not synthesize or release DA under normal conditions their role in the regulation of prolactin secretion is unknown. In contrast to the DM-ARC, the rostrocaudal distribution

of TH-IR neurons in the VL-ARC is sexually dimorphic (Cheung et al., 1997). In males, there are approximately half to a third fewer numbers of TH-IR neurons in rostral than caudal regions of the VL-ARC. In females, the number of TH-IR neurons is similar throughout the rostrocaudal extent of the VL-ARC and is consistently lower than in males. Thus, a prominent population of TH-IR neurons is present in the VL-ARC of males that is either absent or undetectable in females. There is no sexual difference in the actual numbers of TH-IR neurons expressing FRA in the VL-ARC, but due to greater numbers of TH-IR neurons in males, a lower percentage of these neurons express FRA than in females (Cheung et al., 1997). It is interesting to note that TH-IR neurons in the VL-ARC are most prominent during the neonatal period, but diminish in numbers by puberty (Daikoku et al., 1986). This is likely due to a decrease in the synthesis of TH rather than loss of neurons, since these neurons reappear following anterolateral deafferentation of the mediobasal hypothalamus (Daikoku et al., 1986). These 'DOPAergic' neurons are highly susceptible to the neurotoxic effects of monosodium glutamate (Bodnar et al., 2001).

2.2.2. Incertohypothalamic DA neurons (A_{13})

The IHDA neurons located in the most rostral portion of the MZI were originally described as the A_{13} cell group by Dahlström and Fuxe (1964). Perikarya of these densely packed TH-IR neurons have extensive dendritic processes oriented in the ventral plane which extend into the dorsomedial nucleus of the hypothalamus (Chan-Palay et al., 1984; van den Pol et al., 1984). Early reports using relatively insensitive histochemical fluorescence techniques suggested that efferents of IHDA neurons project diffusely into the surrounding anterior, dorsomedial and posterior regions of the hypothalamus (Björklund and Lindvall, 1984). The results of more recent immunohistochemical tract-tracing (Wagner et al., 1995) and neurochemical studies (Eaton et al., 1994) suggest, however, that IHDA neurons project much more extensively than originally believed, innervating a variety of anatomically discrete brain regions including the central nucleus of the amygdala, horizontal diagonal band of Broca and hypothalamic paraventricular nucleus. The relative contribution of IHDA neurons to these regions varies; i.e. DA terminals in the paraventricular nucleus originate predominantly from IHDA neurons in the MZI, whereas IHDA neurons provide only a minor portion of the DA innervation of the amygdala and horizontal diagonal band of Broca (Cheung et al., 1998). The majority of DA input to these latter two regions originates from the midbrain.

While little information is available regarding the function of IHDA neurons, the distribution of their axonal projections to divergent limbic and hypothalamic brain regions suggests that they may function in the integration of autonomic and neuroendocrine responses to specific sensory stimuli. Indeed, IHDA neurons are located in the most rostral extent of the zona incerta, a diencephalic region involved in processing afferent 'sensory' information and integrating efferent 'motor' responses (Ma et al., 1997). This region receives input from a variety of brain regions involved in sensory processing including the thalamus, hypothalamus, and brain stem reticular formation, and has somatotopically arranged output to all levels of the neuroaxis (Romanowski et al, 1985), including the limbic system and hypothalamus (Wagner et al., 1995; Cheung et al., 1998).

2.2.3. Periventicular-hypophysial (tuberohypophysial) DA neurons (A_{14})

The DA axons terminating in the intermediate and neural lobes of the posterior pituitary were postulated initially to constitute a distinct tuberohypophysial DA neuronal system originating from A_{12} perikarya located in the most rostral extent of the ARC (Björklund and Lindvall, 1984). More recent tract-tracing immunohistochemical studies (Kawano and Daikoku, 1987; Goudreau et al., 1992, 1995) reveal that DA neurons terminating in the intermediate lobe of the posterior pituitary originate from a sub-population of A_{14} DA neurons located in the periventricular nucleus dorsal to the retrochiasmatic area of the anterior hypothalamus rather than the ARC. Indeed, neonatal administration of monosodium glutamate, which destroys A_{12} DA neurons in the DM-ARC (Meister, 1991) but spares PeVDA neurons (Daikoku et al., 1986), results in loss of TH-IR (Daikoku et al., 1986) and DA (Dawson et al., 1985) in the median eminence, but not in the posterior pituitary. The PHDA neurons have dendrites oriented in the dorsoventral plane (Seroogy et al., 1988) and axons that project ventrally through the internal layer of the median eminence and pituitary stalk to terminate in close proximity to intermediate lobe melanotrophs. The DA released from PHDA neurons tonically inhibits the secretion of α-melanocyte stimulating hormone (αMSH; Goudreau et al., 1992) and other proopiomelanocortin (POMC)-derived peptides from melanotrophs in the intermediate lobe (Millington and Chronwall, 1989).

The origin of TH-IR fibers innervating the neural lobe of the posterior pituitary is not clearly defined due, in part, to the difficulty in limiting diffusion of injected retrograde tracer into the intermediate lobe (Kawano and Daikoku, 1987). DA innervation of the neural lobe is of central origin since DA is completely absent following pituitary stalk transsection, whereas superior cervical ganglionectomy has no effect on neural lobe DA concentrations (Saavedra, 1985). DA neurons terminating in the neural lobe have been reputed to originate in the rostral ARC (Björklund et al., 1973), periventricular nucleus (Kawano and Daikoku, 1987), ventrolateral retrochiasmatic area in sheep (Gayrard et al., 1995), and interestingly, the paraventricular nucleus, colocalized in magnocellular neurons located in these regions (Young et al., 1987). Little information is available regarding the function of DA in the neural lobe of the posterior pituitary, but water deprivation-induced vasopressin secretion is accompanied by a marked increase in neural lobe DA concentrations (Manzanares et al., 1990) suggesting a role in osmotic regulation of vasopressin secretion. Dependent upon the experimental model employed, DA and/or DA receptor agonists have been reported to have either no effect (Pitzel and Konig, 1984), stimulate (Gálfi et al., 2001), or inhibit (Lightman et al., 1982) vasopressin release via a direct action in the neural lobe. The stimulatory effect of DA on vasopressin release from in vitro cultures which lack viable magnocellular neuronal axon terminals may be due to a direct action on non-neuronal vasopressin-synthesizing pituicytes (Gálfi et al., 2001). Details regarding the DA regulation of posterior pituitary hormone secretion are included in Section 4.1.3.

2.2.4. Periventricular hypothalamic DA neurons (A_{14})

Perikarya of PeVDA neurons are distributed in the periventricular nucleus throughout the entire rostrocaudal extent of the third ventricle (Chan-Palay et al., 1984; van den Pol et al., 1984). Dendrites of these neurons are oriented in the dorsoventral plane and overlap extensively with dendrites from adjacent IHDA neurons. PeVDA perikarya are also

distributed along the ventral surface of the brain near the suprachiasmatic nucleus (van den Pol et al., 1984). In the anteroventral region of the periventricular nucleus, the distribution of PeVDA neurons is sexually dimorphic in that the number of TH-IR cells and fibers is 2 to 3-fold higher in females than in males (Simerly et al., 1985a). Masculinization of PeVDA neurons in this region occurs during the early neonatal period since testosterone treatment of female pups up to postnatal day 5 results in a reduction in the numbers of TH-IR neurons in adults to that characteristic of males (Simerly et al., 1985b). This effect of testosterone is likely due to its conversion to estrogen since genetically altered male mice which lack estrogen receptors develop a phenotypic female distribution of TH-IR neurons in this region (Simerly et al., 1997). Conversely, testicular feminized male mice which lack androgen receptors have a similar distribution of TH-IR neurons as wild type control males (Simerly et al., 1997). Although little information is available regarding the projections of PeVDA neurons, the fibers of these neurons in the rostral periventricular nucleus extend laterally into the adjacent medial preoptic nucleus and anterior hypothalamic area (Björklund and Lindvall, 1984). PeVDA neurons (along with other chemically identified neuronal populations) in the rostroventral periventricular nucleus are components of a gonadal steroid responsive sexually dimorphic forebrain circuitry believed to mediate preovulatory gonadotropin secretion in females (Simerly, 1995) and copulatory behavior in males (Hull et al., 1997, 1999).

2.2.5. Ventrolateral hypothalamic DA neurons (A_{15})

Perikarya of A_{15} DA neurons were originally described in rats as comprising two distinct groups; a compact dorsal group located in the ventral portion of the bed nucleus of the stria terminalis that extends caudally and medially to a position below the anterior commissure, and a long ventrolateral group located above the optic chiasm near the supraoptic nucleus beginning at the level of the preoptic area and extending caudally through the retrochiasmatic area (Hökfelt et al., 1984). Like A_{12} TH-IR neurons in the VL-ARC, these neurons lack DDC and under normal conditions synthesize DOPA rather than DA (Hökfelt et al., 1984; Mons et al., 1990). The A_{15} ventrolateral neurons are bipolar with long neuronal processes that extend into the lateral hypothalamus and caudal borders of the supraoptic nucleus (Mons et al., 1990). Retrograde tract-tracing studies performed in rats reveal that both dorsal and ventral groups of A_{15} neurons innervate the supraoptic nucleus suggesting a role in the regulation of oxytocin and/or vasopressin secretion via an action on magnocellular soma located in this region (Van Vulpen et al., 1999; see Section 4.1.3).

In seasonal breeding species such as sheep (Tillet and Thibault, 1989) and cattle (Leshin et al., 1995), the dorsal A_{15} cell group is absent. The ventrolateral A_{15} cell group is comprised of a heterogeneous population of DA immunopositive and DA immunonegative TH-IR neurons (Tillet et al., 1990) that project ventromedially through the internal layer of the median eminence to the neural lobe of the posterior pituitary (Gayrard et al., 1995). These neurons receive synaptic contacts from noradrenergic efferents originating in the A_1 cell group in the ventrolateral medulla (Tillet and Thibault, 1993; Tillet et al., 2000) and have numerous glial processes in close apposition to their soma and dendrites (Tillet and Thibault, 1993). It has been postulated that these processes may control noradrenergic synaptic input to these neurons during various endocrine states. The role of A_{15} ventrolateral DA neurons in the regulation of luteinizing hormone secretion in seasonal breeders will be discussed later in this chapter.

2.3. DIENCEPHALIC DA NEURONS AND AGING

The majority of studies on the effects of the aging process on diencephalic DA neurons have focused on TIDA neurons in the rat, especially with respect to the consequences of age-related changes in their activity on circulating levels of prolactin. Serum concentrations of prolactin increase as rats age, in part, because of diminution in inhibitory DA control of prolactin secretion (Demarest et al., 1980) and development of prolactin secreting anterior pituitary tumors (Phelps et al., 1987). While there is general agreement that the functional activity of TIDA neurons in both male and female rats decreases with age, this is somewhat paradoxical since prolactin (which increases with age) would be expected to feed back and stimulate (rather than inhibit) the activity of TIDA neurons in an attempt to suppress secretion of this hormone. Apparently, the ability of prolactin to activate TIDA neurons is impaired in aged rats and these animals release less DA into the hypophysial portal blood in response to prolactin as compared with younger animals (Gudelsky et al., 1981; Reymond and Porter, 1981; Reymond, 1990). Further details regarding this and other aspects of prolactin feedback regulation of TIDA neurons will be presented later in this chapter.

The inability of the TIDA neurons to suppress chronic hyperprolactinemia in aged animals could be due to degenerative loss of these neurons, but this remains controversial since the numbers of DA perikarya in the ARC have been reported to increase (Selemon and Sladek, 1986), decrease (Voogt et al., 1990) or remain unchanged during aging (Reymond et al., 1984; Phelps et al., 1987). On the other hand, there is uniform agreement that DA stores in axon terminals in the median eminence (Demarest et al., 1980; Estes and Simpkins, 1980, 1984; Hoffman and Sladek, 1980) and DA release into hypophysial portal blood (Gudelsky et al., 1981; Reymond and Porter, 1981) decrease with age. Although gene expression (Kedzierski and Porter, 1990) and mass of TH in the ARC (Aguila-Mansilla et al., 1993) do not decline in TIDA neurons in aged rats, the affinity of TH for its tyrosine substrate and pteridine cofactor is reduced causing insufficient activation of the cyclic AMP-dependent protein kinase A pathway and a resultant deficit in phosphorylation of TH protein (Reymond et al., 1984). Accordingly, impairment of the ability of TIDA neurons to inhibit prolactin secretion with age is likely due to a diminished capacity of TH to synthesize the DA precursor DOPA (Demarest et al., 1980; Reymond et al., 1984), and need not involve permanent loss of these neurons. In mice, DA in the median eminence declines with age, but in this species there is no age-related change in numbers of TIDA neuronal perikarya or development of hyperprolactinemia (Selemon and Sladek, 1981).

There have been fewer studies on age-related changes in the PeVDA and IHDA neuronal systems. There is no evidence of a reduction of numbers of perikarya (Reymond et al., 1984; Selemon and Sladek, 1984; Phelps et al., 1987), but there is a loss of DA content in the terminal region of PHDA neurons in the posterior pituitary (Reymond and Porter, 1981; Estes and Simpkins, 1984). There are, however, no reports on age-related deficits in the functions of these neurons (i.e. changes in circulating concentrations of αMSH or β-endorphin that are normally regulated by these neurons; Tilders et al., 1985). There is a single report on the intensity and numbers of IHDA neurons in the MZI of aging rats (Selemon and Sladek, 1986) showing that these neurons exhibit little alteration in fluorescence intensity during the aging process, but there is a consistent 30–35% reduction in their numbers in 30 month male rats when compared to those in 3 month rats. There is no evidence of a loss of terminals of these neurons in hypothalamic

regions. Because so little is known about the functions that are controlled by IHDA neurons, it is not possible to determine if the age-related loss of these neurons is accompanied by any functional deficit.

3. NEUROCHEMICAL AND MOLECULAR CHARACTERISTICS OF DIENCEPHALIC DA NEURONS

Diencephalic DA neurons share many properties with mesotelencephalic DA neurons that are characteristic of their neurochemical phenotype including similar DA biosynthetic and catabolic enzymes, precursor intermediates, and inactive metabolites. Many of the distinctive regulatory mechanisms of mesotelencephalic DA neurons such as, end-product inhibition of TH enzymatic activity, reuptake transporters, and presynaptic inhibitory DA autoreceptors have also been shown to be present in most (but not all) diencephalic DA neuronal systems. Accordingly, much of what is known regarding the regulation and function of these neurons is based upon analytical techniques and experimental approaches initially applied in seminal studies of the major ascending DA neuronal systems. However, there are several unique aspects of diencephalic DA neurons that preclude exclusive use of certain techniques in the absence of additional corroborative evidence. These are mostly related to the small size of many hypothalamic nuclei innervated by diencephalic DA terminals, the relatively higher density of noradrenergic versus DA innervation in many of these regions, and (in some cases) the presence of dual DA innervation originating from other diencephalic and/or mesotelencephalic DA neuronal systems.

For example, the hypothalamic paraventricular nucleus receives dual DA innervation from neurons in the MZI (Cheung et al., 1998) and local intrinsic PeVDA neurons located in the surrounding neuropil (Liposits et al., 1986). This region is also densely innervated by stress-responsive noradrenergic neurons originating in the hindbrain (Moore and Bloom, 1979). Since catecholamine neurons share common biosynthetic and catabolic enzyme systems, under stimulated conditions, excess newly synthesized DA can be metabolized to 3,4-dihydroxyphenylacetic acid (DOPAC) rather than being converted to norepinephrine in noradrenergic neurons (Tian et al., 1991). This precludes the use of DOPAC alone as a neurochemical index to estimate stress-induced changes in DA neuronal activity in this region without other neurochemical measures or additional information obtained using different techniques. The advantages and disadvantages of using neurochemical and molecular indices of diencephalic DA neuronal activity will be discussed where applicable in the following section.

3.1. NEUROCHEMICAL EVENTS ASSOCIATED WITH DA SYNTHESIS, RELEASE AND METABOLISM IN AXON TERMINALS OF DIENCEPHALIC DA NEURONS

The study of the regulation and function of diencephalic DA neuronal systems has progressed in parallel with the availability of sensitive analytical techniques capable of detecting meaningful changes in DA synthesis and metabolism in discrete brain regions containing axon terminals of these neurons. As described in the previous section, early formaldehyde-based histofluorescence techniques were capable of identifying the location of catecholamine perikarya and their processes (Dahlström and Fuxe, 1964), but

these techniques were relatively insensitive and could not distinguish between DA-, norepinephrine- and epinephrine-containing neurons. With the development of dual immunohistochemistry protocols utilizing specific antibodies for TH, DA-β-hydroxylase, phenylethanolamine-N-methyltransferase, DOPA and DA (used in conjunction with high precision tract-tracing methods) investigators were able to identify the location of diencephalic DA neurons, but they lacked a true in vivo measure of the activity of these neurons. Indeed, only a few studies have attempted to directly measure the activity (or impulse flow) of diencephalic DA neurons. For example, Sanghera (1989) and Eaton and Moss (1989) recorded electrical activity from IHDA neurons in the MZI in response to a variety of pharmacological manipulations using both in situ and in vitro slice preparations. Other laboratories have recorded electrical activity in slices of the mediobasal hypothalamus, particularly from neurons in the ARC (Lin et al., 1993; Liang and Pan, 2001), but only a few studies determined unequivocally that recordings were made from TIDA neurons (Loose et al., 1990; Wagner et al., 1997).

Early attempts to directly measure DA release from diencephalic neurons in rats using microdialysis have been limited by the small size and close proximity of nuclei containing terminals of these neurons, and the necessity of using relatively large probes to detect low levels of extracellular DA in these regions (e.g. Kapoor and Chalmers, 1987; Lavicky and Dunn, 1993; Timmerman et al., 1994). More recent studies have successfully combined smaller microdialysis probes for individual nuclei sample collection with highly sensitive analytical techniques to measure DA in these dialysates (Hull et al., 1995; Ohtani et al., 1999), but microdialysis has not been commonly employed in the study of these neurons. Rather, much of what is known regarding the in vivo activity of diencephalic DA neurons is based upon studies exploiting the coupled relationship between the synthesis, release and metabolism of DA that operates to maintain steady-state levels of DA in axon terminals of these neurons.

As shown in Fig. 3 (Top Panel), dietary tyrosine is transported into axon terminals of DA neurons and converted in the cytoplasm to DOPA by the rate limiting enzyme TH. DOPA is then rapidly decarboxylated by DDC to DA which is taken up and stored in synaptic vesicles until release. Excess newly synthesized DA is metabolized by mitochondrial monoamine oxidase (MAO) to DOPAC which rapidly diffuses out of neurons and is taken up and converted to homovanillic acid (HVA) by catechol-O-methyltransferase (COMT)-containing glial cells in the neuropil (Hansson and Sellström, 1983; Kimelberg, 1986). Upon arrival of an action potential at the axon terminal, vesicular DA is released into the synapse via calcium-dependent exocytosis where it is free to interact with stimulatory D_1 and/or inhibitory D_2 DA receptors on postsynaptic target cells and inhibitory D_2 autoreceptors on presynaptic terminals. A major portion of DA is removed from the synapse by high affinity DA transporters located on presynaptic terminals, and recaptured DA is either metabolized to DOPAC by mitochondrial MAO or stored in synaptic vesicles for subsequent re-release. A small portion of DA can also be taken up from the synapse by glia and metabolized to 3-methoxytyramine (3MT) and HVA.

The TIDA neurosecretory neurons terminating in the median eminence lack true synapses and the DA released from these neurons into the extracellular fluid diffuses through fenestrated capillaries of the hypophysial portal system where it is transported to the anterior pituitary (Fig. 3; Bottom Panel). Considering this unique cytoarchitecture, it is not surprising that the TIDA neurons are reported to lack inhibitory autoreceptors (Demarest and Moore, 1979b; Timmerman et al., 1995a), have lower levels of DA transporter mRNA (Meister and Elde, 1993) and protein (Ciliax et al., 1995; Revay et al.,

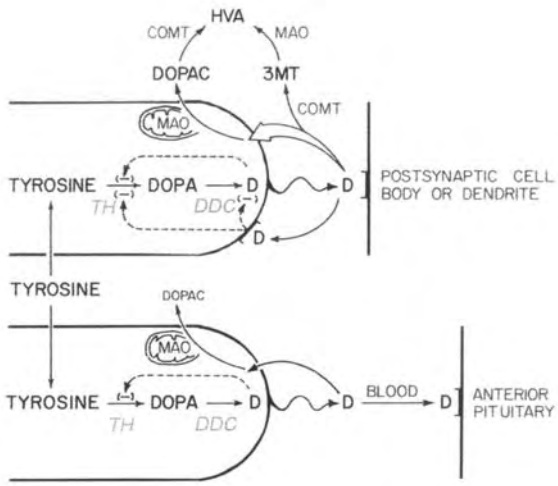

Fig. 3. Schematic representation of the neurochemical events associated with neurotransmitter synthesis, release, re-uptake and metabolism in axons of diencephalic DA neurons terminating in classical synapses (Top Panel), and TIDA neurosecretory neurons terminating in close proximity to the hypophysial portal system (Botton Panel). Arrows with dashed lines represent end-product inhibition of TH activiy by DA (Top + Bottom Panels) or DA presynaptic autoreceptor-mediated inhibition of DA synthesis and release (Top Panel). Abbreviations: COMT, Catechol-O-methyltransferase; D, dopamine; DDC, DOPA decarboxylase; DOPA, 3,4-dihydrophenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; MAO, monoamine oxidase; 3MT, 3-methoxytyramine; TH, tyrosine hydroxylase.

1996; Hoffman et al., 1998), and display only low affinity DA uptake in vitro (Demarest and Moore, 1979a; Annunziato et al., 1980), although this latter point remains controversial (e.g. Sarkar et al., 1983; Plantjé et al., 1987; Garris and Ben-Jonathan, 1991; DeMaria et al., 2000a). In the absence of appreciable DA uptake in vivo, DOPAC (derived mainly from catabolism of newly synthesized, unreleased DA) represents the major measurable DA metabolite in the median eminence (Lookingland et al., 1987a). Nonetheless, impulse-driven release of DA from these and all other diencephalic DA neurons is accompanied by an increase in the synthesis of a new neurotransmitter via a mechanism likely involving loss of DA end-product inhibition of TH activity.

3.2. NEUROCHEMICAL ESTIMATION OF THE ACTIVITY OF DIENCEPHALIC DA NEURONS

Investigators have employed a variety of neurochemical techniques to estimate neurotransmitter release from diencephalic DA neurons. The basis of these methods is that the release of DA is coupled to the rates of synthesis and metabolism of DA in terminals of DA neurons. Procedures that increase or decrease the neurotransmitter release from the DA neurons generally do not alter steady state concentrations of DA, but produce corresponding increases or decreases, respectively, in rates of synthesis, turnover and metabolism of this amine. The utility of various neurochemical procedures for estimating activity of diencephalic DA neurons has been discussed earlier (Moore, 1987a), and only reviewed briefly and updated in this section.

A number of investigators have employed in vitro techniques to characterize neurochemical properties of TIDA and PHDA neurons, but as this chapter will focus

on the responses of diencephalic DA neuronal systems to physiological and pharmacological manipulations, discussions will be limited to results obtained using in vivo and/or ex vivo techniques. Early in vivo attempts to estimate the activity of central catecholaminergic neurons involved studies that employed α-methyltyrosine, an inhibitor of TH. Following administration of α-methyltyrosine the concentrations of catecholamines are reduced in an exponential manner at a rate that is proportional to the activity of the neurons containing these amines. The advantage of this technique is that it permits concurrent estimation of DA and norepinephrine turnover in the same hypothalamic brain region. There are, however, several disadvantages to this procedure: (1) measurement of catecholamines must be made in groups of animals killed immediately before and at least two different times after α-methyltyrosine administration so as to assure that an exponential rate of decline has occurred, (2) rapid measurements cannot be made which prohibits the use of α-methyltyrosine for short term experimental manipulations, and (3) by virtue of its ability to block synthesis α-methyltyrosine reduces catecholamine release which compromises neuronal function. This presents a confounding complication especially when studying prolactin regulation of TIDA neurons since blockade of DA synthesis in TIDA neurons reduces DA release into the hypophysial portal blood thereby removing DA inhibition of prolactin secretion from the anterior pituitary. The increase in circulating prolactin feeds back to increase activity of TIDA neurons even in controls.

The rate of catecholamine synthesis is regulated at the step catalyzed by TH, so that estimates of catecholaminergic activity can be obtained from measurements of the activity of this enzyme. This can be accomplished in vivo by administering 3-hydroxybenzylhydrazine (NSD 1015), an inhibitor of DDC. The concentration of DOPA in brain tissue is essentially zero because once it is synthesized from tyrosine, it is immediately decarboxylated to DA. Following the administration of NSD 1015, DOPA accumulates in catecholaminergic nerve terminals at a rate that is proportional to the activity of these neurons. The advantages of this procedure over the α-methyltyrosine technique are that fewer measurements are needed (DOPA concentrations are so low that 'zero-time' values are unnecessary), and they can be made over a shorter time frame (i.e. as soon as 15 min after i.v. NSD 1015). As with α-methyltyrosine, NSD 1015 disrupts catecholamine synthesis and thereby alters the properties of the catecholaminergic neurons (e.g. NSD 1015, like α-methyltyrosine, increases plasma levels of prolactin). Finally, DOPA accumulates in both DA and noradrenergic neurons after the administration of NSD 1015. This has little consequence when DOPA accumulation is measured in terminals of TIDA and PHDA neurons in the median eminence or intermediate lobe of the pituitary, respectively, since the concentrations and turnover of DA greatly exceed those of NE. In most other hypothalamic regions, however, the concentrations of NE are greater than DA, so this procedure cannot be employed to estimate IHDA, PeVDA or A_{15} ventrolateral DA neuronal activity.

In brain regions containing a preponderance of DA over noradrenergic nerve terminals, the concentrations of the DA metabolite DOPAC reflect the activity of DA neurons. It has been shown empirically that increases and decreases in TIDA and PHDA neuronal activities are accompanied by concurrent increases and decreases in DOPAC concentrations in the median eminence and intermediate lobe of the pituitary, respectively (Lookingland et al., 1987a,b; Lindley et al., 1990a). In contrast to techniques that require administration of α-methyltyrosine or NSD 1015, no drug pretreatments are required prior to the measurement of DOPAC concentrations and measurements can be made immediately after initiating a manipulation.

With some precautions, changes in concentrations of DOPAC and DA can be used to estimate changes in the activities of IHDA and PeVDA neurons despite the relative higher density of NE innervation to some of these regions (Tian et al., 1991). DA is a precursor of NE, and as such is present in low concentrations in noradrenergic neurons. When impulse flow in noradrenergic neurons increases, TH is activated and the synthesis of DA within these neurons increases. Because of limitations imposed by transport of DA into synaptic vesicles and/or the activity of DA-ß-hydroxylase (which is located within these vesicles) DA within noradrenergic neurons accumulates and some of the amine is metabolized to DOPAC (Andén and Grabowska-Andén, 1983; Scatton et al., 1984). Thus, a concurrent increase in both DOPAC and DA concentrations within a region without a significant change in the DOPAC/DA ratio is usually indicative of an increase in the activity of noradrenergic neurons in this region. If this is the case, the increase in DA and DOPAC will be accompanied by an increase in the concentrations of 3-methoxy-4-hydroxy-phenylethylene glycol (MHPG) a major metabolite of NE. An increase in DOPAC without a change in DA concentrations (i.e. increase in DOPAC/DA ratio) usually signifies an increase in DA neuronal activity within a region. In order to substantiate this conclusion, it is advisable to determine that concentrations of MHPG do not change, and to measure DOPAC/DA ratios in brains in which noradrenergic neurons have been destroyed by intracerebral injections of 6-hydroxydopamine (Tian et al., 1991).

3.3. MOLECULAR EVENTS ASSOCIATED WITH SYNTHESIS OF TYROSINE HYDROXYLASE IN PERIKARYA OF DIENCEPHALIC DA NEURONS

Changes in neurochemical activity associated with impulse-driven neurotransmitter release from axon terminals of diencephalic DA neurons are often accompanied by corresponding changes in TH synthesis in regions containing perikarya of these neurons (e.g. Arbogast and Voogt, 1991b). This activity-dependent synthesis of TH is believed to be regulated, in part, through the interaction of dimerized inducible transcription factors with activating protein-1 (AP-1) binding sites located on the TH gene promoter (Icard-Liepkalns et al., 1992). Advances have been made in our understanding of the mechanisms underlying activity-related regulation of TH synthesis in diencephalic DA neurons using dual immunohistochemical and in situ hybridization techniques, but the majority of these studies have been correlative in design and focused mainly on TIDA neurons. Accumulating evidence suggests that regulation of TH gene expression in these neurons is temporally associated with induction of the Fos family of transcription factors. Indeed, the relative number of TH-IR neuronal perikarya in the ARC expressing the immediate early gene products Fos and its related antigens (FRA) is associated with experimentally-induced changes in neurochemical activity (Lerant et al., 1996, 1997, 2001; Cheung et al., 1997; Hentschel et al., 2000) and TH gene expression in TIDA neurons (Wang et al., 1993; Hoffman et al., 1994). There have been no studies, however, demonstrating a causal relationship between FRA expression and TH synthesis in these or any other diencephalic DA neurons.

As depicted in Fig. 4, stimulation of FRA expression involves ligand-mediated activation of membrane receptors located on neuronal perikarya and/or dendrites which causes second messenger-mediated phosphorylation of protein kinases, FRA gene transcription, and synthesis of FRA mRNAs and proteins (Hesketh, 1995; Hughes and Dragunow, 1995). In TH neurons, FRA proteins (including Fos, FRA1, FRA2 and FOSB; Hesketh, 1995) are translocated to the nucleus where they form heterodimers with

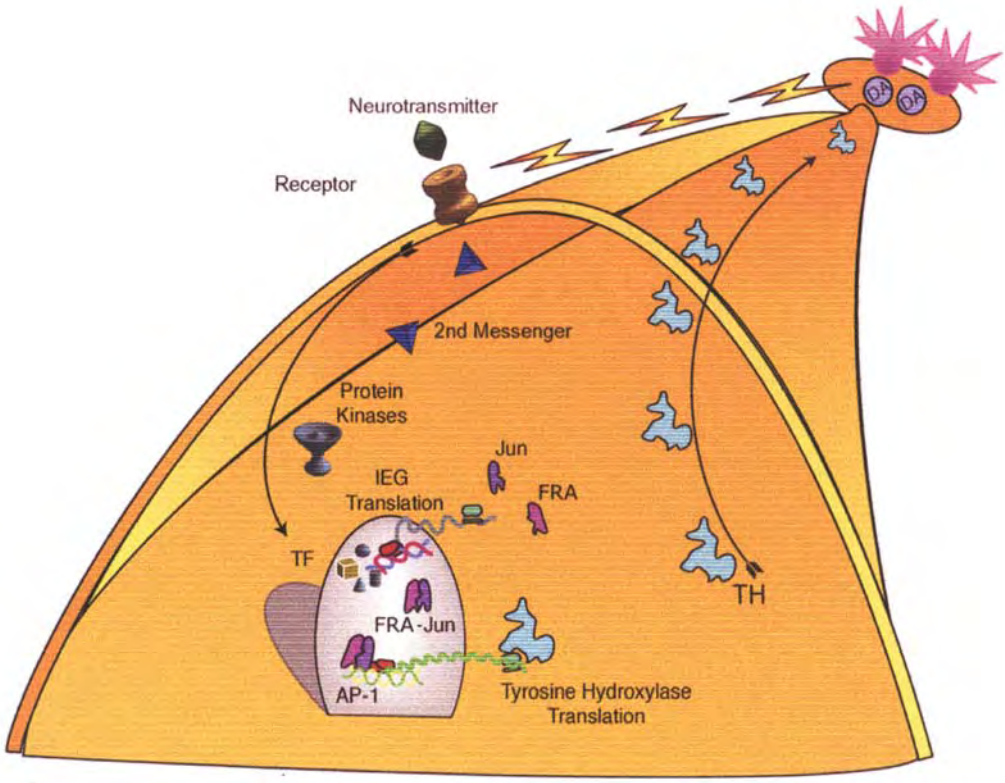

Fig. 4. Schematic depicting the role of FRA immediate-early gene (IEG) transcription factors (TF) in mediating activity-dependent synthesis of tyrosine hydroxylase (TH) in diencephalic DA neurons.

constitutively expressed Jun-related transcription factors that bind to the AP-1 promoter site on the TH gene and facilitate transcription of TH mRNA (Icard-Liepkalns et al., 1992). In neonatal cell cultures derived from mixed populations of diencephalic TH-IR neurons both cAMP and calcium mediate induction of Fos (Sim et al., 1994a). In TIDA neurons alterations in FRA precede activity-dependent changes in TH mRNA expression which is consistent with a role for these transcription factors in stimulating TH biosynthesis (Wang et al., 1993; Hoffman et al., 1994). The presence of FRA proteins in nuclei of TH-IR neurons provides a useful cell body marker of activity that permits identification of individual DA neurons in the ARC that participate in coordinated DA release from axon terminals in the median eminence in response to modality-specific stimuli (Hoffman and Murphy, 2000).

Only a limited number of studies have examined responses of other diencephalic DA neuronal systems using inducible immediate early gene mapping of FRA proteins and expression of TH mRNA, but with few exceptions (e.g. Lerant et al., 1996) these have mostly served as negative controls in experimental paradigms designed to study responses of TIDA neurons (e.g. Arbogast and Voogt, 1991a,b; Bot and Chahl, 1998; Lerant et al., 2001) or hindbrain noradrenergic neurons (Sagar et al., 1995). Nonetheless, the results of these few studies have revealed that IHDA, PeVDA and PHDA neurons all express Fos, FRA, and TH mRNA, but no information is available regarding regulation of expression

of these gene products in these neurons. The results of these studies are discussed later in the relevant sections of this chapter.

3.4. DA RECEPTOR-MEDIATED REGULATION OF DIENCEPHALIC DA NEURONS

Central DA receptors were originally divided into two pharmacologically distinct subtypes on the basis of their biochemical effects on adenylyl cyclase activity (Kebabian and Calne, 1979). D1 receptors activate adenylyl cyclase; whereas D2 receptors inhibit this enzyme. More recent ligand binding and molecular cloning studies have established multiple variants of these receptors which form two separate families, the D1-like and D2-like DA receptors (Civelli et al., 1993). The D1-like family is composed of two subtypes (D_1 and D_5) which are predominantly distributed as postsynaptic receptors (Richtand et al., 1995), whereas the D2-like family consists of three subtypes (D_2, D_3 and D_4) which function both as pre- and postsynaptic receptors (Richtand et al., 1995). The discovery of multiple subtypes of the DA receptor has led to the development of second generation agonists and antagonists selective for D1- and D2-like DA receptors with utility for studying the role of these receptors in the regulation of central DA neurons and their neurological functions.

Results from early studies using DA receptor agonists and antagonists that do not distinguish between D1 and D2 receptor families demonstrated that IHDA neurons are regulated by DA receptor-mediated mechanisms (Moore, 1987a), and in this respect resemble DA neurons comprising the mesotelencephalic neuronal systems. Indeed, non-selective DA receptor agonists, such as apomorphine decrease, whereas DA receptor antagonists, such as haloperidol increase the activity of IHDA neurons. Local application of DA inhibits the firing rate of neurons in the MZI by activating inhibitory autoreceptors on IHDA perikarya or dendrites (Eaton and Moss, 1989; Sangera, 1989). These effects are likely mediated by D_3 (or possibly a subtype of D_2) DA receptors since IHDA neurons are responsive to the mixed D_2/D_3 antagonist raclopride, but not the selective D_2 antagonist remoxipride (Eaton et al., 1992). Selective activation of D_2 (but not D_1) receptors blocks stimulated Fos expression in neonatal cell cultures derived, in part, from TH-IR neurons in the MZI. This suggests an inhibitory role for DA autoreceptors in the regulation of immediate early gene expression in IHDA neurons (Sims et al., 1994b).

The PeVDA neurons are also regulated by DA receptor-mediated mechanisms (Moore, 1987a). Acute administration of the DA receptor antagonists and agonists increase and decrease, respectively, the activity of these DA neurons in the periventricular nucleus and adjacent medial preoptic nucleus and anterior hypothalamic area. Furthermore, inhibition of neuronal activity following administration of gamma-hydroxybutyrolactone results in an apomorphine-reversible increase in DA concentrations in these regions suggesting that PeVDA neurons are regulated, at least in part, by DA autoreceptors (Moore, 1987a). PHDA neurons terminating in the intermediate lobe of the posterior pituitary are also responsive to DA agonists and antagonists, whereas DA neurons terminating in the neural lobe are not (Lookingland et al., 1985). In this respect, neural lobe DA neurons resemble TIDA neurons terminating in the median eminence.

Although early pharmacological studies demonstrated that TIDA neurons are not regulated by inhibitory D_2 autoreceptors (Moore and Lookingland, 1995), these neurons are responsive to the acute administration of selective D_1 and $D_{2/3}$ receptor agonists. Indeed, acute administration of DA agonists with preferential affinity for $D_{2/3}$ receptors

(i.e. quinpirole and quinelorane) stimulates TIDA neurons (Berry and Gudelsky, 1991; Eaton et al., 1993), through an action at D_2 (rather than D_3) receptors (Durham et al., 1997). This stimulatory action of $D_{2/3}$ agonists occurs via an afferent neuronal mechanism involving inhibition of tonically active dynorphinergic interneurons (Durham et al., 1996). The inability of DA antagonists to alter the activity of TIDA neurons per se suggests that there is little intrinsic endogenous DA agonism of the D_2 receptor under basal conditions (Eaton et al., 1993). Conversely, acute administration of D_1 agonists (e.g. SKF 38393, CY 208-243) inhibits both 'basal' (Durham et al., 1998) and 'activated' TIDA neurons (Berry and Gudelsky, 1990). The opposing actions of stimulatory D_2 and inhibitory D_1 receptors could account for the net lack of effect of mixed D_1/D_2 agonists on TIDA neurons (Durham et al., 1998).

Acute administration of 'classical' antipsychotics with D_2 receptor antagonistic properties (e.g. haloperidol) activates DA neurons that comprise the mesotelencephalic systems, but has no direct action on TIDA neurons. On the other hand, these neurons are activated indirectly several hours after administration of haloperidol and other D_2 antagonists as a result of their ability to increase circulating concentrations of prolactin (Moore, 1987b). By contrast, some atypical neuroleptics, exemplified by clozapine, increase acutely TIDA neuronal activity (Gudelsky and Meltzer, 1989). Although it has been proposed that this action of clozapine involves interactions with D_1 and/or neurotensin receptors, the mechanism by which clozapine increases the activity of TIDA neurons remains to be elucidated. This action of clozapine may, however, be responsible for the drug's brief elevation of plasma prolactin levels compared to the long duration of its other effects. That is, clozapine's ability to increase release of DA from TIDA neurons may counteract it's relatively weak D_2 antagonistic actions thereby causing only transient prolactin secretion from anterior pituitary lactotrophs.

4. DA REGULATION OF PITUITARY HORMONE SECRETION

Diencephalic DA neurons were originally implicated in the regulation of pituitary hormone secretion on the basis of the results of early receptor binding and pharmacological studies showing that: (1) DA receptors are located in hypophysiotropic regions of the hypothalamus and pituitary gland, and (2) activation or blockade of these receptors alters pituitary hormone secretion both in vivo and in vitro (Moore, 1987a). These findings (along with those demonstrating the presence of DA terminals in regions of the hypothalamus and posterior pituitary containing perikarya or axon terminals of neurosecretory neurons) suggested that diencephalic DA neurons may control pituitary hormone secretion by a direct action on DA receptors located on pituitary endocrine cells, or indirectly through regulation hypothalamic inhibitory and/or stimulatory neuropeptide release into the hypophysial portal blood.

4.1. DIRECT ACTION OF DA ON HORMONE SECRETING CELLS IN THE PITUITARY

The direct actions of DA on pituitary hormone secretion are largely inhibitory, maintaining basal secretion of prolactin from anterior pituitary lactotrophs and POMC-derived peptide hormones from intermediate lobe melanotrophs via inhibitory D_2 receptors located on these cells. Episodic surges of these hormones are associated with

both loss of DA receptor-mediated inhibition of hormone secretion (secondary to inhibition of diencephalic DA neurons) and direct activation of endocrine cells by stimulatory secretogogues. DA also inhibits growth hormone (GH) and thyrotropin release via direct actions in the anterior pituitary, but under normal baseline conditions these DA systems are not tonically active. Because DA regulation of these hormones occurs mainly through actions on hypothalamic neurosecretory neurons, these hormones are discussed in the next section (see Section 4.2). In the case of vasopressin (and possibly oxytocin), diencephalic DA neurons have been implicated in the stimulation of hormone secretion, but these neurons may also function to dampen activated hormone release under certain physiological conditions. These apparently divergent actions are likely due to differential effects of multiple DA neuronal systems on magnocellular neuronal cell bodies in the hypothalamus as opposed to axon terminals in the neural lobe of the posterior pituitary.

4.1.1. Prolactin

The primary function of TIDA neurons in both the females and males is to suppress the secretion of prolactin from the anterior pituitary. Indeed, experimental procedures that: (1) disrupt synthesis and release of DA from TIDA neurons in the median eminence, (2) prevent access of DA to the anterior pituitary lactotrophs (i.e. pituitary stalk section; pituitary transplantation), or (3) block pituitary D_2 receptors, all increase prolactin secretion in both sexes (Ben-Jonathan, 1985). In females, the inhibitory effect of DA on the synthesis and secretion of prolactin is opposed by estrogen, and as a consequence circulating prolactin levels in gonadally-intact females are higher than males. Higher basal prolactin in females, in turn, tonically stimulates TIDA neurons such that under normal conditions the activity of these neurons is 2–3 times that of males (Moore and Lookingland, 1995). This higher set point of basal TIDA neuronal activity in females may be physiologically relevant for regulation of episodic hormone secretion since prolactin surges which occur during proestrous, pregnancy, lactation and stress are all associated with suppression of TIDA neuronal activity and loss of DA inhibition of prolactin secretion (Moore and Lookingland, 1995; Freeman et al., 2000; Ben-Jonathan and Hnasko, 2001). Details regarding prolactin regulation of TIDA neurons and neuronal disinhibition of the activity of these neurons that facilitates prolactin secretion during proestrous, pregnancy, lactation and stress will be discussed later in this chapter.

There is also evidence that DA neurons terminating in the posterior pituitary may participate in the regulation of prolactin secretion under basal conditions and during physiological states in females that are associated with episodic hormone release (for reviews see Ben-Jonathan et al., 1991; Freeman et al., 2000; Ben-Jonathan and Hnasko, 2001). Disruption of DA input to the posterior pituitary following either surgical lobectomy or stalk denervation increases basal prolactin secretion in both male and cycling female rats, and this is reversed by peripheral infusion of DA (Ben-Jonathan and Hnasko, 2001). It is believed that the DA originating from neurosecretory neurons in the neural lobe is transported to the anterior pituitary via short portal vessels that traverse (but do not communicate with) the relatively avascular intermediate lobe (Baertschi, 1980). It is unlikely that PHDA neurons terminating in the intermediate lobe participate directly in regulation of prolactin secretion from anterior pituitary lactotrophs because DA released from these neurons is rapidly removed from the synapse by DA transporters

located on their axon terminals (Garris and Ben-Jonathan, 1991; DeMaria et al., 2000a). On the other hand, suckling-induced inhibition of PHDA neurons may facilitate prolactin surges indirectly via an αMSH-dependent mechanism (Frawley, 1994; Vecsernyés et al., 1997). αMSH increases the sensitivity of pituitary lactotrophs to prolactin-releasing factors and recruits additional prolactin-synthesizing cells into the active secretory pool (Frawley, 1994).

4.1.2. αMSH and β-endorphin

αMSH and multiple acetylated forms of β-endorphin are the predominant POMC-derived peptide hormones secreted by melanotrophs in the intermediate lobe of the posterior pituitary gland (Akil et al., 1984; Millington and Chronwall, 1989). The synthesis and release of these hormones is tonically suppressed by DA acting on inhibitory D_2 receptors located directly on these cells (Coté et al., 1982; Tilders et al., 1985; Millington and Chronwall, 1989). Pharmacological blockade of post-synaptic D_2 receptors (which displaces and prevents binding of endogenous DA to receptors) increases: (1) expression of POMC mRNA (Chronwall et al., 1988), (2) post-translational processing and secretion of POMC-derived peptides (Penny and Thody, 1978; Tilders et al., 1985; Millington et al., 1987; Lindley et al., 1988), and (3) the rate of proliferation of intermediate lobe melanotrophs (Chronwall et al., 1987). Compelling evidence indicates that the suppressive effects of DA are mediated by D_2 receptor inhibition of adenylyl cyclase and lowering of intracellular cAMP in melanotrophs (Millington and Chronwall, 1989). Secretion of POMC-derived hormones is also regulated by stimulatory β_2-adrenergic receptors which oppose the actions of DA, activate adenylyl cyclase and increase intracellular cAMP (Coté et al., 1982; Tilders et al., 1985; Kvetnansky et al., 1987). Pharmacological activation of D_2 receptors blocks the stimulatory effect of β_2-adrenergic receptor activation on secretion in vitro (Munemura et al., 1980; Coté et al., 1982; Meunier and Labrie, 1982) and in vivo (Lindley et al., 1990b), suggesting that DA is the predominant regulator of cAMP levels in these cells.

DA innervation of the intermediate lobe originates from PHDA neurons located in the hypothalamic periventricular nucleus (Goudreau et al., 1995). The axons from these neurons course through the median eminence and pituitary stalk to the intermediate lobe where they make direct synaptic contact with melanotrophs (Holzbauer and Racké, 1985). DA is measurable in the intermediate lobe by the 4th day of life when an abundance of melanotrophs are already present in this gland (Gary and Chronwall, 1992). Differentiation of axon terminals of PHDA neurons is regulated by trophic factors secreted by intermediate lobe melanocytes (Charli et al., 1993), and DA released from these neurons, in turn, suppresses melanotroph proliferation and heterogeneity (Millington and Chronwall, 1989). In adults, experimental procedures that increase or decrease DA release from PHDA neurons cause reciprocal changes in αMSH secretion both in vivo (Lindley et al., 1988) and ex vivo (Davis, 1986). There are no sexual differences in DA concentrations in the intermediate lobe (suggesting a similar density of DA innervation of melanotrophs in females and males), but the rate of DA synthesis and metabolism are slightly higher in females as compared with males (Manzanares et al., 1992a). This difference in PHDA neurons is not due to circulating gonadal hormones since neither gonadectomy nor steroid hormone treatment of gonadectomized rats has any effect on the neurochemical activity (Gunnet et al., 1986; Manzanares et al., 1992a) or FRA expression in these neurons (Lerant and Freeman, 1997).

During stress, the release of αMSH from melanotrophs is stimulated as the result of two concurrent events: (1) release of epinephrine from the adrenal medulla which, in turn, activates β_2 adrenergic receptors on melanotrophs and stimulates αMSH secretion, and (2) removal of tonic D_2 receptor-mediated inhibition of hormone secretion exerted by DA released from PHDA neurons (Lindley et al., 1990b). Stress-induced inhibition of PHDA neurons is mediated by histaminergic, serotoninergic and γ-aminobutyric acid (GABA)-ergic neurons, the latter two may be arranged in series. Accumulating evidence indicates that PHDA neurons receive a convergence of inhibitory inputs which are important for removing the tonic inhibition of melanotroph secretion during stress. Further details regarding neuronal regulation of PHDA neurons during stress will be covered later in this chapter.

4.1.3. Vasopressin and oxytocin

Magnocellular vasopressinergic and oxytocinergic neurons located in the hypothalamic paraventricular and supraoptic nuclei project axons, via the paraventricular-supraopticohypophysial pathway to the neural lobe of the posterior pituitary. These neurons are unique in that they represent the only hypothalamic neurosecretory cells that release neurohormones directly into the systemic circulation which, in turn, act directly at peripheral non-endocrine target tissues. The release of neurohormones from these neurons is controlled by neuroendocrine reflex mechanisms that result in the rapid transduction of electrical impulses into hormonal secretory responses. This occurs in the absence of inhibitory peripheral hormone feedback regulation characteristic of hormones secreted by the anterior pituitary. Rather, inactivation of these neurons involves intrinsic cellular mechanisms such as depletion of readily releasable neurohormone pools and changes in membrane calcium permeability (Shaw et al., 1983), as well as extrinsic neuronal, paracrine (and perhaps autocrine) inhibitory regulatory systems.

In the case of vasopressin, hyperosmotic, hypovolumetric and hypotensive stimuli (relayed from central osmoreceptors, and peripheral cardiovascular volume and baroreceptors, respectively) activate magnocellular neurons, via multisynaptic modality-specific neuronal pathways (Sladek, 1983). The circulating vasopressin acts in the distal convoluting tubules in the kidney to facilitate water reabsorption and restrict urine volume (Robertson et al., 1976), and in arterioles to contract smooth muscle cells and increase blood pressure (Share, 1988). Oxytocin release is initiated by peripheral sensory receptors located in the nipple and uterus (Challis and Lye, 1994; Wakerley et al., 1994). Sensory signals are relayed by ascending spinal and hindbrain multisynaptic neuronal pathways which terminate in close proximity to magnocellular neurons in the paraventricular and supraoptic nuclei (Cunningham and Sawchenko, 1991). The circulating oxytocin acts in the mammary gland to contract myoepithelial cells and cause milk letdown during lactation (Wakerley et al., 1994), and in the uterus to contract the myometrium and initiate parturition (Challis and Lye, 1994). Neuronal input to the paraventricular and supraoptic nuclei regulates both the rate and pattern of magnocellular neuronal depolarization via a complex interaction of multiple chemically-identified neurotransmitter systems (Renaud and Bourque, 1991).

The regulation of vasopressin secretion by DA is multifaceted, likely involving participation of one or more separate populations of DA neurons under different physiological situations. Although the possibility exists that extrahypothalamic DA neurons may be involved in reflex activation of vasopressin release (Cornish et al., 1997),

results of studies performed both in vitro and in vivo indicate that diencephalic DA regulation of vasopressin occurs through actions on both magnocellular neuronal perikarya in the hypothalamus and axon terminals in the neural lobe of the posterior pituitary. These studies also reveal that the responsiveness of vasopressin neurons to DA (or its agonists) may differ depending upon the level of secretory activity of magnocellular neurons and whether DA released from diencephalic DA neurons acts at stimulatory D_1 or inhibitory D_2 postsynaptic receptors.

DA innervation of the paraventricular and supraoptic nuclei arises from several distinct populations of diencephalic DA neurons, but little information is available ascribing a definitive role for any of these systems in the regulation of vasopressin secretion. The paraventricular nucleus receives DA input from IHDA neurons in the MZI (Wagner et al., 1995; Cheung et al., 1998) and local intrinsic PeVDA neurons (van den Pol et al., 1984), while the supraoptic nucleus is innervated by adjacent A_{15} ventrolateral DA neurons (Van Vulpen et al., 1999) and possibly PeVDA neurons located below the ventromedial nucleus (Lindvall et al., 1984; van den Pol et al., 1984). The DA axon terminals have been observed in close proximity to magnocellular neurons in both of these nuclei (Buijs et al., 1984). DA neurons terminating in the neural lobe of the posterior pituitary are neurosecretory in nature and found in close proximity (Pelletier, 1983), but do not make direct synaptic contact with vasopressinergic axon terminals (Holzbauer et al., 1983). These DA neurons may originate from several sources including THDA neurons in the rostral ARC (Björklund et al., 1973), PeVDA neurons in the periventricular nucleus dorsal to the retrochiasmatic area (Kawano and Daikoku, 1987), and (in sheep) A_{15} neurons in the ventrolateral retrochiasmatic area (Gayrard et al., 1995).

Early in vitro pharmacological studies demonstrated that DA (acting at different DA receptor subtypes present within the neural lobe; De Souza, 1986; Mansour et al., 1990) has both stimulatory and inhibitory effects on vasopressin release depending upon the rate of neurohormone secretion at the time of DA or DA agonist treatment. Indeed, under basal conditions (when release is low) activation of DA receptors increases vasopressin secretion (Bridges et al., 1976) and vasopressin-associated neurophysin gene expression (Mathiasen et al., 1996); effects believed to be mediated by stimulatory D_1 receptors (Racké et al., 1986; Mathiasen et al., 1996). On the other hand, electrically-evoked vasopressin secretion is suppressed by DA (Lightman et al., 1982) via a mechanism involving inhibitory D_2 receptors (Racké et al., 1986). Blockade of DA receptors has no effect per se, but prevents both the stimulatory and inhibitory effects of DA on vasopressin secretion (Bridges et al., 1976; Lightman et al., 1982; Racké et al., 1986). DA may inhibit vasopressin release via D_4 receptor-mediated presynaptic inhibition of excitatory glutamatergic input to magnocellular neuronal perikarya (Price and Pittman, 2001). Taken together, these results suggest that DA (released from THDA, PeVDA and/or A_{15} ventrolateral DA neurons in the neural lobe) may regulate vasopressin secretion by initiating neurohormone release from axon terminals of quiescent neurons and (during activated conditions) dampen excessive vasopressin release via actions in the neural lobe.

Interestingly, the magnocellular vasopressinergic neurons express TH mRNA during chronic stimulation (Young et al., 1987) suggesting the possibility that these neurons are capable of synthesizing and releasing DA in response to prolonged activation which may act in an autocrine fashion in the neural lobe to either enhance or dampen vasopressin secretion. Indeed, several days of dehydration-induced vasopressin secretion is accompanied by increases in DA synthesis (Alper et al., 1980) and DA concentrations in the

neural lobe without affecting levels of the DA metabolite DOPAC (Manzanares et al., 1990a). The inability of prolonged hyperosmotic stimuli to alter neural lobe DOPAC is inconsistent with the hypothesis that increased DA synthesis occurs in terminals of DA neurons since changes in the activity of these neurons are accompanied by corresponding changes in DA metabolism (Lindley et al., 1990a). Rather, the increase in DA in the absence of a change in DOPAC suggests the possibility that DA is synthesized in vasopressin neurons in the neural lobe and protected from deamination by mitochondrial MAO by storage in synaptic vesicles. The functional significance of de novo synthesis of DA within vasopressin neurosecretory neurons in the neural lobe following prolonged hyperosmotic stimuli remains to be elucidated.

Numerous in vivo studies performed in a variety of species have demonstrated that blockade of DA receptors following central or systemic administration of DA antagonists has no effect on basal secretion of vasopressin (e.g. Kendler et al., 1978; Brooks and Claybaugh, 1982; Ivanyi et al., 1986; Yamaguchi et al., 1988). On the basis of these results it is generally believed that release of vasopressin from magnocellular neurons in the neural lobe is not tonically inhibited by diencephalic DA neurons as are other pituitary hormones like prolactin (by TIDA neurons) and αMSH (by PHDA neurons). On the other hand, experimental evidence indicates that DA may act within the brain (as it does in the neural lobe) to facilitate (and possible mediate) neuronal reflex activation of vasopressin secretion. In vivo evidence for a central stimulatory role of DA in the regulation of vasopressin release came from studies showing that intracerebroventricular (icv) injections of either DA (Ivanyi et al., 1986; Yamaguchi et al., 1988) or a DA agonist (Kimura et al., 1981) rapidly increases plasma vasopressin concentrations, an effect prevented by prior administration of a DA antagonist (Ivanyi et al., 1986). That direct injection of low doses of DA into the paraventricular nucleus mimics the stimulatory effect of icv injections of DA on vasopressin secretion (Yamaguchi et al., 1992) suggests that IHDA and/or local intrinsic PeVDA neurons terminating in this region participate in activation of vasopressin release via an action at targeted DA receptors located on or near perikarya of magnocellular neurons. These diencephalic DA neurons may be an integral part of modality-specific activating neuronal circuits involved in vasopressin release in response to angiotensin II (Brooks and Claybaugh, 1982; Yamaguchi et al., 1988) and noxious (but not osmotic) stimuli (Onaka et al., 1992; Yamaguchi et al., 1996). Central DA receptors implicated in osmotic stimulation of vasopressin secretion are located in the 'osmosensitive' anteroventral third ventricular regions (Yamaguchi et al., 1996), suggesting a stimulatory role for PeVDA neurons in more rostral regions of the periventricular nucleus in this process. In agreement, icv injection of either DA or a DA agonist to alcohol-treated (Moos and Richard, 1982) or water-loaded rats (Forsling and Williams, 1984) with depressed vasopressin secretion causes a rapid increase in vasopressin release and inhibition of diuresis.

There is also experimental evidence that central DA neurons may be involved in inhibiting activated vasopressin secretion. In normal hydrated rats icv administration of DA suppresses anesthesia-induced vasopressin secretion (Forsling and Williams, 1984). Conversely, icv injection of a DA antagonist enhances vasopressin secretion in response to hemorrhage (Yamaguchi et al., 1990). No information is availability, however, regarding the identity of diencephalic neurons mediating the suppressive effects of DA on stimulated vasopressin release.

On the basis of the results of early pharmacological studies it is generally believed that DA stimulates spontaneous release of oxytocin and facilitates suckling-induced reflex

activation of hormone secretion during lactation (Poulain and Wakerley, 1982). More recent studies have also shown that DA may function to tonically suppress oxytocin release during periods of non-suckling in lactating female rats (Crowley et al., 1987, 1991). Indeed, systemic or central administration of DA or a DA agonist increases basal oxytocin secretion in both males (Melis et al., 1990; Cameron et al., 1992) and lactating females (Bridges et al., 1976; Moos and Richard, 1982), and blockade of DA receptors prevents suckling-induced oxytocin release (Clarke et al., 1979; Moos and Richard, 1982; Crowley et al., 1991). In males, the stimulatory effect of DA on oxytocin secretion is mediated by inhibitory $D_{2/3}$ receptors (Amico et al., 1993; Uvnas-Moberg et al., 1995), suggesting that DA may act indirectly to stimulate hormone release by suppressing neurotransmitter release from inhibitory interneurons. In females, DA may directly activate oxytocin secretion via stimulatory D_1 receptors on magnocellular perikarya in the paraventricular and supraoptic nuclei (Mason, 1983; Parker and Crowley, 1992) or on axon terminals in the neural lobe (Crowley et al., 1991). The identity of specific diencephalic DA neuronal systems that participate in the regulation of oxytocin secretion is not known.

4.2. INDIRECT ACTION OF DA VIA HYPOTHALAMIC NEUROSECRETORY NEURONS

DA control of anterior pituitary hormone secretion also mediated through transynaptic regulation of hypothalamic neurosecretory neurons. This occurs via axonal–somatic/dendritic interactions in hypothalamic regions containing neurosecretory neuron perikarya and/or through axonal–axonal interactions on their terminals in the median eminence. Diencephalic DA neurons may regulate neuropeptide release directly via stimulatory D_1 or inhibitory D_2 receptors located on hypothalamic neurosecretory neurons, or they may act indirectly through stimulatory and/or inhibitory interneurons.

4.2.1. Gonadotropins

DA regulation of gonadotropin secretion is controversial, especially with regard to the identification of diencephalic DA neuronal systems that participate in control of luteinizing hormone secretion under specific physiological and pathological states. Indeed, depending upon the experimental approach and the animal model employed, the results from early studies have supported both stimulatory and inhibitory roles for DA in the control of gonadotropin releasing hormone (GnRH) release and luteinizing hormone secretion. Due to the lack of information regarding the anatomy of other diencephalic DA neurons, these divergent actions have been attributed to TIDA neurons in the median eminence (Fuxe et al., 1972; Baraclough and Wise, 1982; Kalra and Kalra, 1983). The DA regulation of gonadotropin secretion is likely more complex than originally postulated, involving several DA neuronal systems which regulate GnRH neurons (either directly or indirectly, via interneurons) at anatomically distinct sites.

GnRH neuronal perikarya implicated in the regulation of luteinizing hormone secretion are distributed throughout the rostral diencephalon and adjoining telencephalon, with the high densities found within the horizontal diagonal band of Broca and medial preoptic nucleus (Kalra and Kalra, 1983). Axons of GnRH neuronal perikarya in the horizontal diagonal band course caudally through the mediobasal hypothalamus and terminate in the lateral external layer of the median eminence in close proximity to terminals of TIDA neurons (Ajika, 1979; Merchenthaler et al., 1980; Ugrumov et al., 1989b). On the basis of

this anatomical relationship, these DA neurons have been implicated in mediating hyperprolactinemia-induced suppression of luteinizing hormone secretion (Selmanoff, 1981), but their role in regulating episodic luteinizing hormone secretion is controversial (Kalra and Kalra, 1983). Rather, evidence suggests that IHDA neurons located in the MZI may be important in regulating both proestrus (MacKenzie et al., 1988; Sanghera et al., 1991) and gonadal steroid-induced luteinizing hormone surges (MacKenzie et al., 1984). The finding that IHDA neurons innervate the horizontal diagonal band is consistent with this hypothesis (Eaton et al., 1994; Wagner et al., 1995; Cheung et al., 1998).

GnRH neuronal perikarya in the medial preoptic nucleus also project to the median eminence (Merchenthaler et al., 1980), but the medial preoptic nucleus is innervated by PeVDA neurons located in the adjacent periventricular nucleus (Björklund et al., 1973, 1975; van den Pol et al., 1984; Horvath et al., 1993), rather than by IHDA neurons (Horvath et al., 1993; Wagner et al., 1995; Cheung et al., 1998). Early correlative studies of luteinizing hormone secretion and the activity of DA neurons in the medial preoptic nucleus have been inconclusive (Weiner and Ganong, 1978; Kalra and Kalra, 1983), due, in part, to the failure of these studies to account for changes in the synthesis and metabolism DA which occur in activated noradrenergic neurons terminating in this region (Tian et al., 1991).

Luteinizing hormone pulse frequency during seasonal anestrus in sheep is slower than during estrus due, in part, to a greater sensitivity of GnRH neurons to estrogen feedback inhibition (Legan et al., 1977). A role for DA in mediating estrogen inhibition of luteinizing hormone secretion is supported by the findings that pharmacological blockade of DA receptors increases luteinizing hormone pulse frequency in anestrous estrogen-treated ewes, whereas DA or DA agonist activation of DA receptors suppresses elevated luteinizing hormone pulse frequency observed in the absence of estrogen (Havern et al., 1994). Several lines of evidence suggest that A_{15} ventrolateral DA neurons participate in the estrogen-induced suppression of luteinizing hormone secretion in anestrous sheep. Indeed, estrogen administration to ovariectomized ewes during anestrus stimulates multiunit neuronal activity (Goodman et al., 2000) and DA synthesis, release and metabolism in the lateral retrochiasmatic area (Gayrard et al., 1994; Thiéry et al., 1995), and this is associated with increased expression of FRA in A_{15} ventrolateral DA neurons (Lehman et al., 1996). Moreover, lesions of A_{15} DA perikarya attenuate estradiol-induced suppression of luteinizing hormone secretion in ovariectomized anestrous ewes (Havern et al., 1994). The excitatory effects of estrogen on A_{15} ventrolateral DA neurons are not direct since these neurons do not contain estrogen receptors (Skinner and Herbison, 1997). It is more likely that estrogen acts through estrogen-receptor containing neurons located in the ventromedial preoptic area (Anderson et al., 2001) which send axonal projections to the lateral retrochiasmatic area that regulate the activity of A_{15} ventrolateral DA neurons (Cutter et al., 2001).

4.2.2. Growth hormone

Regulation of growth harmone (GH) secretion in mammals involves a complex interaction between inhibitory (somatostatin) and stimulatory (GH releasing hormone; GHRH) neuropeptides synthesized and released by neurosecretory neurons terminating in the median eminence (Tuomisto and Männistö, 1985; McMahon et al., 2001). Somatostatin and GHRH are transported in the hypophysial portal blood to the anterior pituitary

where they act directly on target receptors on somatotrophs to regulate GH release (Tuomisto and Männistö, 1985). The GH secretion is pulsatile in nature, displaying an ultradian rhythm that corresponds with the oscillatory reciprocal release of somatostatin and GHRH from the hypothalamus (Tannenbaum and Ling, 1984; Plotsky and Vale, 1985). Regulation of the GH secretion varies depending upon an animal's stage of development, age, gender, body composition, nutritional status, and sleep:awake cycle (Thorner et al., 1995; Veldhuis, 1996; McMahon et al., 2001). Numerous neurotransmitters (including DA) have been identified as possible regulators of the GH release during these various physiological states, mainly through actions on somatostatin and/or GHRH neurosecretory neurons (Weiner and Ganong, 1978; Müller, 1989; Bertherat et al., 1995; McMahon et al., 2001).

The primary function of hypothalamic somatostatin neurons is to inhibit GH secretion from the anterior pituitary gland (Brazeau et al., 1973), but the widespread distribution of this neuropeptide suggests that somatostatin may also act as a neurotransmitter or neuromodulator within the brain (Epelbaum, 1986). The most prominent population of hypothalamic somatostatin-containing neurons is located in the periventricular nucleus midline to the anterior hypothalamic areas (Epelbaum et al., 1981; Finley et al., 1981; Johansson et al., 1984). A vast majority (i.e. 70% or more) of these neurons project to the median eminence (Makara et al., 1983; Kawano and Daikoku, 1988; Merchenthaler et al., 1989) and inhibit GH release through a direct action on pituitary somatotrophs (Epelbaum, 1986; Ishikawa et al., 1987). Periventricular somatostatin neurons also project locally within discrete regions of the hypothalamus (Bennett-Clarke et al., 1980; Hisano and Daikoku, 1991; Moga and Sapir, 1994), but the function of these neurons is not clear. Axon terminals of somatostatin neurons in the ARC are found in close proximity to perikarya of neurosecretory GHRH neurons (Willoughby et al., 1984; Liposits et al., 1987; Epelbaum et al., 1989; Bertherat et al., 1992) suggesting that somatostatin may act indirectly to inhibit GH secretion by preventing GHRH release from the hypothalamus (Bertherat et al., 1995; McMahon et al., 2001). GHRH neurons projecting to the median eminence originate in the ARC and (to a lesser extent) perifornical dorsolateral hypothalamic area (Jacobowitz et al., 1983; Merchenthaler et al., 1986a). Interestingly, GHRH neurons in the VL-ARC also contain TH and constitute a subpopulation of A_{12} 'DOPAergic' neurons located in this region (Meister and Hökfelt, 1988; Sakanaka et al., 1990a).

A DA control of the GH secretion occurs in a variety of species through direct actions on anterior pituitary somatotrophs and indirectly via regulation of hypothalamic somatostatin and GHRH neurosecretory neurons (Yamaushi et al., 1991; McMahon et al., 2001). The direct actions of DA on the GH release are mediated by inhibitory D_2 receptors located on somatotrophs (Goldsmith et al., 1979), and in this respect resemble DA inhibition of prolactin secretion from anterior pituitary lactotrophs. The potency of DA on hormone release differs between these two cells types, such that DA inhibits prolactin at concentrations much lower than those needed for suppression of GH (Cronin et al., 1984; Ishibashi and Yamaji, 1984). This relative insensitivity of somatotrophs to DA suggests that DA inhibition of GH release from these cells may only come into play under conditions when supranormal levels of DA are present in the anterior pituitary such as during chronic hyperprolactinemia-induced activation of TIDA neurons (Agrasal et al., 1988).

DA and its agonists inhibit both basal and GHRH-stimulated GH hormone release from the anterior pituitary in vitro (Cronin et al., 1984; Lindström and Ohlsson, 1987),

and these effects are blocked by selective D_2 (but not D_1) receptor antagonists (Ishibashi and Yamaji, 1984). The DA agonists are equally effective as somatostatin in inhibiting the GH release from cultured adenomatous pituitary cells obtained from acromegalic patients (Ishibashi and Yamaji, 1984), and on this basis selective orally-active D_2 receptor agonists are currently under investigation as alternatives to injectable somatostatin analogs in the treatment of acromegaly (Cozzi et al., 1998). The TIDA neurons located in the DM-ARC are likely responsible for direct DA inhibition of GH secretion since DA released from these neurons into the hypophysial portal vasculature represents the major source of DA in the anterior pituitary gland. The finding that TIDA neurons are responsive to the central administration of GH (Andersson et al., 1983) is consistent with this hypothesis and suggests that GH may feed back under certain conditions to regulate its on secretion via an action on TIDA neurons. It is unlikely that TIDA neurons tonically inhibit GH release from somatotrophs under normal conditions since pharmacological blockade of D_2 receptors has no consistent effect on GH secretion in vivo during gestation (Marti-Henneberg et al., 1981) or in adults (Gunnett and Moore, 1988; Cunha-Filho et al., 2001).

Central DA control of GH secretion is predominantly inhibitory, mediated by D_1 receptor stimulation of hypothalamic somatostatin neurons (McMahon et al., 2001). Indeed, pharmacological activation of D_1 receptors selectively increases FRA expression in somatostatin neurons in the periventricular nucleus (McMahon et al., 1998), stimulates somatostatin secretion from hypothalamic slices in vitro (West et al., 1997a), and inhibits both basal and GHRH-stimulated GH secretion in vivo (McMahon et al., 1998). The inability of selective D_1 receptor antagonists to alter GH secretion (Grodum et al., 1998) suggests that D_1 receptors do not tonically stimulate somatostatin neurons or suppress GH release under basal conditions. D_1 receptor activation also inhibits hypothalamic GHRH release via a mechanism involving somatostatin receptors (West et al., 1997b) suggesting that a subpopulation of 'DA responsive' periventricular somatostatin neurons functions to constrain GHRH neurons in the ARC (Bertherat et al., 1995; West et al., 1997b; McMahon et al., 2001). While the identity of the diencephalic DA neuronal system that regulates somatostatin neurons is not known, based on their anatomical location in the periventricular nucleus it is likely that PeVDA neurons are involved. The finding that the neurochemical activity of PeVDA neurons is increased under experimental conditions associated with elevated somatostatin release is consistent with this hypothesis (Gaynor et al., 1995).

On the basis of early pharmacological studies DA has also been reported to have stimulatory effects on GH secretion in vivo (Weiner and Ganong, 1978; Tuomisto and Männistö, 1985), presumably via stimulation of hypothalamic GHRH release (Casanueva et al., 1981; Chihara et al., 1986). It should be noted, however, that many of these studies employed relatively non-specific drugs which did not discriminate between DA and noradrenergic neurons (e.g. Edén et al., 1979; Casanueva et al., 1981), and considering the well documented stimulatory effects of adrenergic receptors on GHRH and GH release (Weiner and Ganong, 1978; Tuomisto and Männistö, 1985; McMahon et al., 2001) many of norepinephrine actions on GH secretion may have been erroneously attributed to DA. On the other hand, in the absence of somatostatin DA has stimulatory effects on both GHRH release in vitro (Kitajima et al., 1989) and GH secretion in vivo (Kakucska and Makara, 1983) suggesting that DA is a secretagogue for both somatostatin and GHRH, but that the GHRH-stimulating action of DA is overridden by its action on somatostatin (Kitajima et al., 1989).

4.2.3. Thyrotropin

Regulation of thyrotropin secretion from the anterior pituitary occurs through direct stimulation by thyrotropin releasing hormone (TRH) released from hypothalamic neurosecretory neurons, and feedback inhibition by elevated levels of circulating thyroid hormones (i.e. thyroxine and triiodothyronine) secreted by the thyroid gland (Morley, 1981). Hypophysiotropic neurosecretory TRH neurons located in the parvocellular subdivision of the paraventricular nucleus project ventrally through the lateral retrochiasmatic area and terminate in the median eminence (Brownstein et al., 1982; Palkovits et al., 1982). The TRH released from these neurons is transported in the hypophysial portal blood to the anterior pituitary where it acts on specific target receptors located on thyrotrophs to stimulate thyrotropin secretion (Gershengorn and Osman, 1996). Thyrotropin stimulates the synthesis and release of thyroid hormones from the thyroid gland which, in turn, inhibit both TRH release from the hypothalamus and thyrotroph responsiveness to the stimulatory actions of TRH (Morley, 1981). Hormonal feedback inhibition of the hypothalamic-pituitary-thyroid axis maintains relatively constant levels of circulating thyroid hormones necessary for prenatal and prepubertal growth and development (Bernal and Nunez, 1995; Oppenheimer and Schwartz, 1997; Anderson, 2001), and maintenance of basal metabolic rate in adults (Muñoz and Bernal, 1997). Cold temperature overrides feedback inhibition of thyroid hormone secretion by activating TRH release from the hypothalamus via multisynaptic neuronal pathways controlled by thermoreceptors located in the preoptic area and anterior hypothalamus (Ferguson et al., 1984; Arancibia et al., 1996). Several chemically-identified hypothalamic neurotransmitters have been implicated in the inhibition of TRH and thyrotropin secretion including DA and somatostatin (Morley, 1981; Tuomisto and Männisto, 1985; Arancibia et al., 1996).

Although somewhat controversial (e.g. Annunziato et al., 1979; Felt and Nedvídková, 1982; Price et al., 1983), the DA regulation of thyrotropin secretion is generally believed to be inhibitory, occurring through the direct actions on the DA receptors located on pituitary thyrotrophs (Goldsmith et al., 1979) and indirectly, via suppression of the TRH release from the hypothalamus. Indeed, DA and DA agonists (albeit in high doses) inhibit thyrotropin release from cultured pituitary cells in vitro (Foord et al., 1983; Dieguez et al., 1984) and attenuate both TRH- and cold-induced thyrotropin secretion in vivo (Krulich et al., 1977; Tuomisto and Männistö, 1985). These inhibitory effects of DA on thyrotropin secretion are blocked by both peripheral and central acting DA receptor antagonists (Tuomisto and Männistö, 1985; Gunnet and Moore, 1988). Administration of DA receptor antagonists alone has no consistent effect on thyrotropin secretion in vivo suggesting that DA does not tonically suppress hormone secretion under normal basal conditions (Gunnet and Moore, 1988). Rather, DA may participate in thyroid hormone-induced feedback inhibition of thyrotropin secretion and/or neuronally-mediated suppression of activated hormone release following cessation of stimuli such as cold temperature. These actions of DA may be mediated by somatostatin-induced inhibition of TRH release from the hypothalamus or direct somatostatin inhibition of thyrotropin secretion from the anterior pituitary gland (Morley, 1981; Arancibia et al., 1996). If this is the case, then the PeVDA neurons terminating in close proximity to perikarya of neurosecretory somatostatin neurons in the periventricular nucleus and/or TIDA neurons terminating near somatostatin axon terminals in the median eminence could be implicated in this process.

There have been several attempts to identify the diencephalic DA neuronal system that participates in the regulation of thyrotropin secretion. The approach used in these studies has been limited to examining the effect of thyroidectomy (alone or in combination with thyroid hormone replacement) on neurochemical estimates of the activity of the TIDA neurons since these neurons represent the major source of DA present in the anterior pituitary gland. The results of these experiments have been contradictory showing a wide range of responses including no change (Brown et al., 1972), enhanced (Reymond et al., 1987; Wang et al., 1994; Yang and Pan, 1994) or suppressed (Andersson and Eneroth, 1985, 1987) activity of TIDA neurons following thyroidectomy. When thyroidectomy-induced changes in TIDA neuronal activity were observed, these were consistently reversed by thyroid hormone replacement (Andersson and Eneroth, 1985, 1987; Reymond et al., 1987; Wang et al., 1994; Yang and Pan, 1994). It should be noted, however, that thyroid hormones inhibit both synthesis and release of prolactin (Mauer, 1982a,b) suggesting the possibility that prolonged thyroidectomy-induced hyperprolactinemia could stimulate TIDA neurons independent of a direct action of thyroid hormones. Consistent with this conclusion is the observation that TIDA neuronal activity is better correlated with circulating prolactin as opposed to thyrotropin following thyroidectomy (Yang and Pan, 1994). On the other hand, thyrotropin administration to hypophysectomized rats increases the activity of TIDA neurons terminating in the median eminence (Andersson et al., 1980, 1985) suggesting that either thyrotropin or thyroid hormones (stimulated by exogenous thyrotropin administration) activate TIDA neurons via a prolactin-independent mechanism.

On the basis of the ability of the TRH to stimulate prolactin secretion (Ben-Jonathan et al., 1989; Samson and Mogg, 1989) there have been several studies examining the effects of TRH and stable TRH analogues on DA release from TIDA neurons both in vitro (Sharp et al., 1982; Kabayama et al., 1986; Nishikawa et al., 1993; Brunetti et al., 2000) and in vivo (Andersson et al., 1985; Ikegami et al., 1992; Timmerman et al., 1995b). The underlying hypothesis in these studies is that TRH may act in parallel to diminish DA inhibition of prolactin by suppressing neurotransmitter release from TIDA neurons while at the same time directly stimulating prolactin secretion via an action on target receptors on pituitary lactotrophs. Transient DA antagonism has been shown to potentiate TRH-induced prolactin secretion both in vitro and in vivo (Martinez de la Escalera and Weiner, 1988; Pan and Wang, 1989; Haisenleder et al., 1991). But, with the exception of one study (Brunetti et al., 2000), TRH has consistently been shown to stimulate (rather than inhibit) both basal and potassium-induced DA release from TIDA neurons (Sharp et al., 1982; Kabayama et al., 1986; Nishikawa et al., 1993) revealing that inhibition of TIDA neuronal activity during phasic release of prolactin is not due to TRH. TRH-induced activation of DA release from TIDA neurons likely occurs through axonal–axonal interactions in the median eminence since central administration of TRH increases DA metabolism in this brain region (Ikegami et al., 1992). The observations that TRH nerve terminals make intimate contact with terminals of TIDA neurons in the median eminence (Nakai et al., 1983) and TRH increases TIDA neuronal activity in hypophysectomized rats is consistent with this conclusion (Andersson et al., 1985). It has been postulated that TRH-induced stimulation of DA release from TIDA neurons counteracts TRH-induced prolactin secretion thereby providing the underlying mechanism for pulsatile prolactin secretion that occurs during pregnancy (Timmerman et al., 1995b) and lactation (Grosvenor and Mena, 1980). Details regarding the regulation of TIDA neurons during phasic release of

prolactin during proestrous, pregnancy, lactation and stress is discussed in Section 7 of this chapter.

4.2.4. Adrenocorticotropin

Regulation of adrenocorticotropin secretion from the anterior pituitary occurs through direct stimulation by corticotropin releasing hormone (CRH) released from hypothalamic neurosecretory neurons, and feedback inhibition by the elevated levels of circulating glucocorticoids (i.e. corticosterone, cortisol) secreted by the adrenal cortex (Tsigos and Chrousos, 1994). CRH neurons are widely distributed throughout the brain with the highest density of perikarya in the hypothalamic paraventricular nucleus and other discrete extrahypothalamic regions including the central nucleus of the amygdala (Swanson et al., 1983). The majority of CRH axons, originating in the parvocellular paraventricular nucleus project to the median eminence where CRH is released and transported in the hypophysial portal blood to the anterior pituitary. CRH stimulates release of adrenocorticotrophin which, in turn, stimulates glucocorticoid secretion from the adrenal cortex. In the absence of stress, the spontaneous activity of the neurosecretory CRH neurons is regulated by glucocorticoid negative feedback inhibition, and stimulatory neuronal afferents mediating the circadian rhythmic activity of these neurons (Whitnall, 1993). In the central nucleus of the amygdala, CRH neurons comprise an independent fiber system which projects via a descending amygdalofugal pathway through the bed nucleus of the stria terminalis and lateral hypothalamus to terminate in brainstem nuclei important in the regulation of the autonomic nervous system (Moga and Gray, 1985; Sakanaka et al., 1986). Collectively, these central CRH neurons are believed to mediate many of the homeostatic responses of the endocrine and sympathetic nervous systems to stress and inflammation (Dunn and Berridge, 1990; Owens and Nemeroff, 1991; Chrousos, 1995; Habib et al., 2001).

The stimulatory effect of the modality-specific stressors on the central CRH neurons has been well documented (Johnson et al., 1992; Pacák and Palkovits, 2001), with substantial evidence suggesting that separate populations of CRH neurons mediate the endocrinological as opposed to the cardiovascular and behavioral responses to stress (Fisher, 1989; Dunn and Berridge, 1990). In the paraventricular nucleus, a subpopulation of vasopressin-containing neurosecretory CRH neurons primarily mediates stress-induced activation of glucocorticoid secretion (Whitnall, 1989), and aminergic neurons including norepinephrine- (Mezey and Palkovits, 1991; Pacák et al., 1992), serotonin- (Fuller, 1990) and histamine-containing (Kjær et al., 1992) neurons have all been postulated to play a role in this process. Although a role of DA in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis during stress has been largely discounted (e.g. Leibowitz et al., 1989), a more recent experimental evidence suggests that diencephalic DA neurons innervating the paraventricular nucleus may play a stimulatory role in the regulation of the HPA axis. Under what physiological and/or pathological conditions these DA neurons mediate activation of the HPA axis is not known.

The diencephalic DA neurons do not tonically regulate spontaneous (and perhaps rhythmic) activity of the HPA axis since pharmacological blockade of DA receptors has no effect on CRH mRNA expression in the paraventricular nucleus (Zhou et al., 2001), or secretion of adrenocorticotropin (Borowsky and Kuhn, 1992) or glucocorticoids into the circulation (Fuller and Snoddy, 1984). On the other hand, diencephalic DA neurons may participate in neuronal stimulation of the HPA axis since pharmacological activation of

either D_1 or D_2 receptors stimulates expression of Fos and CRH mRNA in CRH neurons in the paraventricular nucleus (Eaton et al., 1996), and elevates secretion of both adrenocorticotropin (Ježova et al., 1985; Borowsky and Kuhn, 1992) and glucocorticoids (Kitchen et al., 1988; Borosky and Kuhn, 1992). The stimulatory effects of DA agonists on the HPA axis are prevented by prior administration of selective DA antagonists (Ježova et al., 1985; Kitchen et al., 1988; Borowsky and Kuhn, 1992; Eaton et al., 1996) connoting an action on central DA receptors (Holland et al., 1978; Ježova et al., 1989). The demonstration that injection of small doses of a DA uptake inhibitor into the paraventricular nucleus mimics the stimulatory effects of this drug following systemic injection (Borowsky and Kuhn, 1993; Kuhn and Francis, 1997) implicates an action on DA neurons terminating in this region. Two diencephalic DA neuronal systems project to the paraventricular nucleus. The PeVDA neurons in the dorsal periventricular nucleus project laterally into the adjacent parvocellular paraventricular nucleus (van den Pol et al., 1984; Liposits and Paull, 1989) and, in turn, receive axosomatic synapses from CRH neurons in this region (Thind and Goldsmith, 1989). The paraventricular nucleus is also innervated by DA axons of IHDA neurons located in the MZI suggesting that these neurons could also participate in the regulation of CRH neurosecretory neurons (Wagner et al., 1995; Cheung et al., 1998). That central CRH administration increases the metabolism of DA in the hypothalamus (Dunn and Berridge, 1987) and, in particular, the paraventricular nucleus (Pan et al., 1995) suggests that CRH neurons may, in turn, regulate the activity of PeVDA and/or IHDA neurons projecting to this region. In view of the widespread use of DA agonists and antagonists in the treatment of neurological disorders further investigations into the role of diencephalic DA neurons in the regulation of the HPA axis seems warranted.

In the central nucleus of the amygdala, CRH neurons are important in the integration of autonomic (Brown and Gray, 1988), behavioral (Lee and Sung, 1989) and (possibly) immunological (Irwin, 1994) responses to stress. While little information is available regarding the neurochemical identity of afferent neurons which regulate these CRH neurons under stressful conditions, a role for norepinephrine (Kiss and Aguilera, 1992) and serotonin neurons (Owens et al., 1990) has been proposed. Although IHDA neurons project to central amygdala (Wagner et al., 1995; Cheung et al., 1998) and DA axons terminate in close proximity to CRH neurons in this region (Hornby and Piekut, 1989), it is unlikely that these or any other central DA neurons are involved in activation of these CRH neurons since they are unresponsive to acute administration of DA receptor agonists (Eaton et al., 1996).

5. HORMONAL REGULATION OF DIENCEPHALIC DA NEURONS

Studies on hormonal feedback regulation of diencephalic DA neurons have mainly focused on the stimulatory effects of prolactin on TIDA neurons and the role of gonadal steroids in determining sexual differences in the development, distribution and activity of these neurons. The results of these experiments are summarized in this section along with comparisons of the effects of various experimental treatments on IHDA, PHDA/THDA and PeVDA neurons. Information on the feedback effects of other hormones such as GH, thyrotropin and thyroid hormones is scant and best described in context of DA regulation of each specific neuroendocrine axis. Accordingly, these have been included in the preceding section.

5.1. PROLACTIN

The TIDA neurons are activated by prolactin. This was first shown by Hökfelt and Fuxe (1972) when they demonstrated that systemic administration of this hormone to rats increased the α-methyltyrosine-induced decline in DA histofluorescence exclusively in the median eminence. Subsequent investigators using quantitative biochemical procedures have demonstrated that systemic and ventricular injections of prolactin increase the activity of TIDA, but not other DA neurons (Moore, 1987b). There is a delay of 12–16 h before the actions of prolactin became evident and at least part of the delay appears to be secondary to processes that involved alterations in gene expression and protein synthesis.

Complicating the results of these early studies was the observation that the drugs used in the biochemical estimations of DA neuronal activity cause hyperprolactinemia. That is, inhibitors of TH (α-methyltyrosine) and aromatic L-amino acid decarboxylase (NSD 1015) reduce DA synthesis in the terminals of TIDA neurons and consequently the amount of DA released into the portal blood (Gudelsky and Porter, 1979). As a result there is a lessening of DA inhibitory control on prolactin release from lactotrophs in the anterior pituitary. The resulting hyperprolactinemia activates the TIDA neurons causing their baseline activity to be high prior to the administration of prolactin. This confounding factor was prevented by conducting experiments in hypophysectomized animals or by pretreating with DA agonists or prolactin antibodies so that circulating levels of endogenous prolactin (and its stimulatory effects on TIDA neurons) remain low prior to the administration of exogenous prolactin. Under these experimental conditions increases in synthesis and turnover of DA in the median eminence can be observed as early as 2–4 h after the administration of prolactin. This initial response is followed some 8–12 h later by a further increase in the activity of TIDA neurons, and only this latter effect is prevented by inhibitors of protein synthesis.

The two phases of prolactin activation of TIDA neurons (rapid 'tonic' and delayed 'induction' components) suggest that the level of activity of these DA neurons reflects both amount of circulating prolactin at the time of measurement (tonic component) and the past history of blood levels of this hormone (induction component). That is, animals treated chronically in such a way as to maintain high circulating concentrations of prolactin exhibit an exaggerated stimulatory response to acute injections of prolactin. Conversely, chronic hypoprolactinemia causes a reduced response to the acute administration of this hormone (Demarest et al., 1985a).

5.1.1. LOCALIZATION OF PROLACTIN RECEPTORS

The realization that prolactin regulates its own secretion by increasing the release of DA from TIDA neurons prompted searches for the site(s) of prolactin receptors (PRL-R) that mediate the short loop feedback circuit. That is, does prolactin activate the TIDA neurons directly, or does it act on other neurons that project to the mediobasal hypothalamus? To answer this question, efforts have been made to characterize and localize PRL-R in the brain. Not surprisingly, since prolactin exerts numerous centrally mediated behavioral and endocrinological actions, PRL-R have been identified throughout the brain (Roky et al., 1996), with dense concentrations in the mediobasal hypothalamus and choroid plexus (Barton et al., 1989; Muccioli et al., 1991; Crumeyrolle-Arias et al., 1993). PRL-R mRNA is also located in the ARC and other brain regions, and two forms of the receptor

(long and short) are expressed in some brain regions (Chiu et al., 1992; Chiu and Wise, 1994; Bakowsky and Morrell, 1997). Arbogast and Voogt (1997) using cultured fetal hypothalamic neurons found PRL-R immunostaining in TH-IR neurons, providing anatomical support for a direct effect of prolactin on TIDA neurons. However, since PRL-R are also located on non-DA neurons within the mediobasal hypothalamus an indirect action of prolactin on TIDA neurons is also possible.

In a more recent in vivo study employing double label immunocytochemistry for PRL-R and TH, Lerant and Freeman (1998) convincingly demonstrated the presence of PRL-R in A_{14} DA perikarya in the periventricular nucleus (PHDA neurons), and A_{12} perikarya in the DM-ARC (TIDA neurons) and VL-ARC (A_{12} DOPAergic neurons) in ovariectomized rats with and without replacement with estrogen and/or progesterone. In contrast, PRL-R were not located in A_{13} perikarya in the MZI (IHDA neurons). These results suggest that in addition to TIDA neurons, other TH-IR neuronal systems originating in the mediobasal hypothalamus may be responsive to short loop negative feedback regulation by prolactin.

The results of early studies revealed that the central PRL-R, and presumably the neurons they regulate, can be modified by changes in the endocrinological milieu. The numbers of PRL-R are higher in the hypothalamus of female than of male rats. Ovariectomy reduces PRL-R and this is reversed by treatment with estrogen (Muccioli et al., 1991). The percentage of hypothalamic DA neurons expressing PRL-R in ovariectomized rats is increased with estrogen replacement and this increase parallels changes in circulating concentrations of prolactin (Lerant and Freeman, 1998). This supports earlier reports by Muccioli and Di Carlo (1994) who found that the numbers of PRL-R are altered in response to changes in serum prolactin levels. That is, hyperprolactinemia (caused by injections of ovine prolactin or a DA antagonist, or by renal implants of anterior pituitaries) and hypoprolactinemia (following DA agonist injections) increases and decreases, respectively, in the numbers of PRL-R in the rat hypothalamus. Changes in distribution of PRL-R may be responsible, at least in part, for changes in TIDA neuronal activity that occur in differing endocrinological states; for example, during the estrous cycle, pregnancy, lactation and suckling, and stress (Sugiyama et al., 1994; Bakowsky and Morrell, 1997; Pi and Gratton, 1999; Grattan, 2001).

5.1.2. Neurotrophic effects of prolactin on TIDA neuronal development

Dwarfism in the mouse is an hereditary trait; the affected mice exhibit retarded postnatal growth, lack of estrous cyclicity, hypothermia and sterility. The primary deficit in the two major types of dwarf mice (Snell and Ames) is in the anterior pituitary, which is deficient in the production and release of prolactin, GH and thyrotropin (for review, see Phelps, 1994). The adult dwarf mice also have a deficit in TIDA neurons; the content and rate of synthesis of DA in the median eminence, and numbers of TH-IR perikarya in the ARC are markedly reduced. This deficit appears selective for TIDA neurons as there is no change in the numbers of DA perikarya in the MZI (IHDA neurons) or substantia nigra (nigrostriatal DA neurons).

Prior to postnatal day 21, the characteristics of the TIDA neurons in dwarf mice cannot be distinguished from those in the normal littermate controls. That is, the DA content in the mediobasal hypothalamus and the number of TH-positive perikarya are the same in dwarf and normal mice. After 21 days of life, TIDA neurons in the dwarf mice regress such that their number at 60 days of age is less than it is at 21 days. This could represent the

death of these DA neurons, or a reduction in their TH content, below the level of detectability. In the same animals nigrostriatal DA neurons continue to develop normally. The abnormal development of the TIDA neurons in dwarf mice appears to result from a deficiency in circulating prolactin during a critical period of development since daily treatment of these mice with prolactin from day 12 through day 60 restores DA histofluorescence in the median eminence and numbers of TH-IR perikarya in the ARC. There is some controversy, however, as to whether or not treatment of the adult dwarf mice with prolactin, fully restores TIDA neuronal function (Phelps, 1994). Replacement of growth hormone or thyroxine is without effect on the loss of TIDA neurons in dwarf mice.

The fact that up to postnatal days 14–21, the TIDA neurons in dwarf mice appear normal may be related to the fact that during the first two weeks of postnatal life, pups receive prolactin from their mother's milk. But, once tight junctions in the intestinal mucosa of the pups develop, large molecules such as prolactin can no longer be absorbed and the pups are deprived of this maternally-derived hormone. Since the anterior pituitaries of the dwarf mice do not secrete prolactin, there is no endogenous hormone to maintain the development of their TIDA neurons. Supporting this proposal are data showing that lowering the prolactin content in the milk of the mother rat by treating the dams with a DA agonist (bromocriptine) on postnatal days 2–5 results in a reduction in the content and turnover of DA in the median eminence, but not in the posterior lobe of the pituitary in the pups (Shyr et al., 1986). The apparent deficit of the TIDA neuronal activity in mice deprived of prolactin as pups is reflected functionally in high circulating levels of this hormone in adults.

Studies employing the Snell and the Ames dwarf mice have revealed the importance of prolactin in maintaining the functional integrity of TIDA neurons. Nevertheless, observations in dwarf mice can be confounded by the fact that they are also deficient in GH and thyrotropin. Studies using another mouse model to determine the consequences of life-long prolactin deficiencies (i.e. mice with targeted disruption of the prolactin structural gene; PRL-KO) have yielded results that are different in one aspect to those obtained in the dwarf mice. Phelps and Horesman (2000) reported that the male and female PRL-KO mice also exhibit reduced DA fluorescence and TH-IR intensity in TIDA neurons in the ARC and median eminence. The male PRL-KO mice also have decreased DA content in the median eminence (Steger et al., 1998). Similar to results in dwarf mice, PRL-KO mice had normal populations of IHDA, PeVDA and nigrostriatal DA neurons. However, unlike in the dwarf mice where the numbers of TH-IR neurons were reduced, the numbers of these neurons in the PRL-KO were the same as in control mice. The results of this study suggest that although prolactin deficiency reduces TIDA neuronal activity, loss of prolactin may not affect differentiation of these neurons. It remains to be determined if GH or thyrotropin can substitute for the trophic developmental effects of prolactin on TIDA neurons in PRL-KO mice.

5.1.3. Prolactin feedback regulation of TIDA neuronal activity

Numerous studies have shown that hyperprolactinemia (resulting from administration of prolactin, DA antagonists or estrogens, or surgical implantation of anterior pituitary glands or prolactin-secreting pituitary tumors) increases the rates of synthesis and turnover of DA in the median eminence and the release of DA into the hypophysial portal blood (Moore, 1987b). On the other hand, these neurochemical indices of DA neuronal activity are reduced by hypoprolactinemia caused by surgical hypophysectomy or

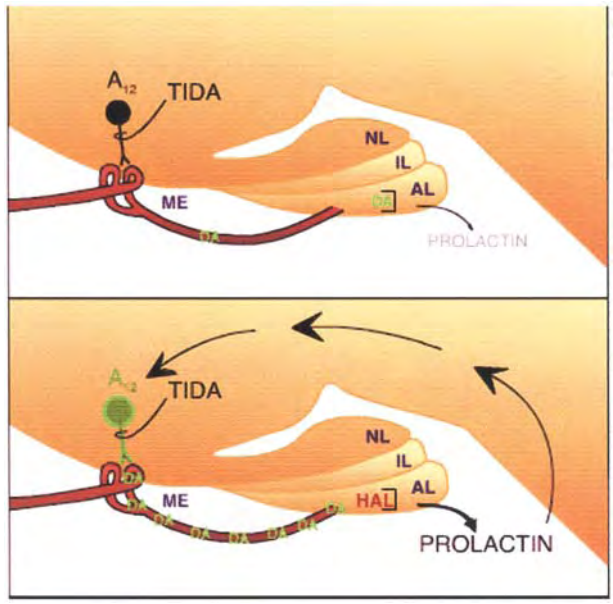

Fig. 5. Parasagittal section through the mediobasal hypothalamus and pituitary of the rat illustrating schematically the regulation of TIDA neurons by prolactin. TOP PANEL: In the resting steady state the secretion of prolactin from anterior pituitary lactotrophs is tonically inhibited by DA originating from TIDA neurons. BOTTOM PANEL: When the resting state is disrupted by blocking the inhibitory actions of DA on the lactotrophs following administration of a DA antagonist (e.g. haloperidol), the release of prolactin increases. The high circulating concentration of prolactin feeds back to activate DA release from TIDA neurons. Abbreviations: AL, anterior lobe; DA, dopamine; HAL, haloperidol; IL, intermediate lobe; ME, median eminence; NL, neural lobe; TIDA, tuberoinfundibular DA neurons.

administration of either DA agonists or prolactin antibodies. Arita and Kimura (1986) measured TH in vitro and showed that the activity of this enzyme was increased in hypothalamic slices from hyperprolactinemic rats, and this increase was not observed when the slices were incubated with tetrodotoxin or in calcium-free media. This suggests that prolactin increases the firing rate of TIDA neurons. The results of these and other studies using a variety of experimental approaches (Selmanoff, 1985) suggest that prolactin exerts a negative feedback on its own secretion by increasing the activity of the inhibitory TIDA neurons. This is depicted schematically in Fig. 5.

References to early studies on the neurochemical responses of TIDA neurons to acute and chronic hyper- and hypo-prolactinemia can be found in review articles (Moore, 1987b; Moore and Lookingland, 1995). More recent studies on this topic have been concerned with the mechanisms by which prolactin influences TIDA neurons (Freeman et al., 2000; Ben-Jonathan and Hnasko, 2001).

5.1.4. Prolactin regulation of tyrosine hydroxylase in TIDA neurons

Studies on the mechanisms by which changes in circulating concentrations of prolactin alter the activation of TIDA neurons have focused on the properties of TH, the enzyme that catalyzes the first and rate-limiting step in the biosynthesis of DA. Catecholaminergic neurons can respond to short-term, acute hormonal signals by rapid, transient changes in the phosphorylation of TH, and to long-term, chronic changes in hormonal signals by

alterations in the synthesis and/or degradation of TH protein (Masserano and Weiner, 1983; Zigmond, 1988/89).

The rapid (i.e. less than 4 h) activation of TH in the median eminence by prolactin that constitutes the 'tonic' component of prolactin stimulation does not require protein synthesis, but is probably associated with effects on the catalytic properties of this enzyme. Pasqualini and coworkers (1994) demonstrated in vitro that prolactin acts directly on TH in the mediobasal hypothalamus to trigger the phosphorylation of this enzyme. This effect, possibly mediated by protein kinase C, makes the enzyme less susceptible to inhibition by newly synthesized DA. That is, prolactin-induced short-term activation of TH results from the removal of end-product inhibition of the enzyme. Conversely, the acute reduction in TH activity measured in vitro in median eminence removed from rats 4 h after administration of bromocriptine is prevented by the coadministration of prolactin (Arbogast and Voogt, 1995). This can also be prevented by an inhibitor of phosphoprotein phosphatases, suggesting that rapid suppression of TH activity secondary to the bromocriptine-induced hypoprolactinemia may also result from dephosphorylation of the enzyme.

The delayed (i.e. 12–16 h) stimulation of TH in the median eminence by the 'induced' component of prolactin activation requires ongoing protein synthesis, possibly of new molecules of TH. This is supported by the findings of Arbogast and Voogt (1991b) who showed that administration of rat or ovine prolactin for 3–7 days increases TH gene expression (TH mRNA) and the amount of TH protein in perikarya of TIDA neurons in the ARC, but not in perikarya of IHDA or nigrostriatal DA neurons in the MZI and substantia nigra, respectively. Conversely, three days of hypoprolactinemia induced by bromocriptine reduced TH mRNA and the amount of TH protein in the ARC, and this effect could be prevented by the concomitant administration of prolactin.

The responses of TIDA neurons to prolactin administration and to experimentally-induced changes in endogenous circulating concentrations of this hormone in intact and castrated male and female rats do not extend to all endocrine states. For example, prolactin-induced activation of TIDA neurons is diminished in rats during late pregnancy (Demarest et al., 1983a) and lactation (Demarest et al., 1983b,c) and after prolonged administration of estrogen (Demarest et al., 1984) and DA antagonists (MohanKumar et al., 1990). A discussion of changes in the cyclical activity of TIDA neurons that occur during the first half of pregnancy and during lactation and suckling can be found in Section 7.

5.2. SEXUAL DIFFERENCES IN THE ACTIVITY OF DIENCEPHALIC DA NEURONS

The basal activity of TIDA neurons and the responsiveness of these neurons to endocrinological, pharmacological and physiological manipulations are different in male and female rats (for review, see Moore, 1987a). Major sex differences are not observed in other hypothalamic DA neurons or in the ascending mesotelencephalic DA neurons. Although the density of TIDA neurons as indicated by the numbers of TH positive perikarya in the ARC (Brawer et al., 1986; Cheung et al., 1997) and DA concentrations in the median eminence (Gunnet et al., 1986; Lookingland et al., 1987a) is the same in male and female rats, the activity of these neurons (as reflected in the rates of synthesis and turnover of DA in the median eminence and concentration of DA in the hypophysial portal blood) is 2–3 times greater in the female (Gudelsky and Porter, 1981). Following

castration TIDA neuronal activity is increased in the male and decreased in the female, and these effects are reversed by testosterone and estrogen replacement, respectively (Gunnet et al., 1986; Toney et al., 1991). The higher level of activity of TIDA neurons in females appears to result from a greater sensitivity to prolactin. A reduction in circulating concentrations of prolactin (as a result of hypophysectomy, or the administration of prolactin antibodies or DA agonists) causes a greater reduction of TIDA neuronal activity in females than in males suggesting that TIDA neurons in females are tonically activated by circulating levels of prolactin, whereas this action is less pronounced in males.

Sexual differences in the TIDA neuronal activity are the result of androgen-induced alterations in the neonatal brain. That is, the activity of TIDA neurons in the adult female rats which were administered testosterone five days after birth was the same as that observed in the adult male, whereas TIDA neuronal activity in adult males who had been castrated on the first day of life exhibited 'female-like' activity (Demarest et al., 1981). Early neurochemical studies determined that there were no sexual differences in the turnover of DA in axon terminals of neurons in either the intermediate or neural lobes of the posterior pituitary gland (Gunnet et al., 1986), but later experiments showed that the synthesis and metabolism of DA in the intermediate lobe were slightly greater in females than in males (Manzanares et al., 1992a). However, this difference in activity of PHDA neurons was not altered by either gonadectomy or gonadal steroid treatment (Manzanares et al., 1992a). Thus, PHDA neurons in the intermediate lobe are not responsive to circulating gonadal steroids as adults and in this respect differ from TIDA neurons. There is no sexual difference in the activity of DA neurons terminating in the neural lobe, and these neurons are unresponsive to gonadectomy and gonadal steroid treatment (Gunnet et al., 1986; Manzanares et al., 1992a). Accordingly, it is unlikely that DA neurons terminating in the neural lobe participate in gonadal steroid regulation of prolactin secretion.

5.2.1. Estrogen

It is generally agreed that short term treatment (1–14 days) with estrogens activates TIDA neurons and this effect is secondary to the ability of these hormones to increase circulating levels of prolactin. That is, results of early studies employing histochemical or neurochemical techniques revealed that injections of estrogens or subcutaneous implants of silastic capsules containing estrogens activated TIDA neurons in intact, but not in hypophysectomized rats (for review, see Moore, 1987b).

Longer treatments with estrogens (more than two weeks) cause a complex pattern of effects on TIDA neurons which have been postulated to result from the direct actions of estrogens per se, hyperprolactinemia, and the encroachment upon the mediobasal hypothalamus by estrogen-induced enlargements or tumors of the anterior pituitary (Moore et al., 1987). A number of studies on the effects of chronic estrogen treatment on TIDA neurons have been conducted in the Fischer 344 rats, a strain that quickly develops prolactin-secreting adenomas and hyperprolactinemia. It is not clear if all of the responses of TIDA neurons to estrogen in this strain of rat are representative of the responses in other strains or species. TIDA neurons in other strains of rats, however, do exhibit sex differences to pharmacological and physiological manipulations. For example, TIDA neurons in female Long Evans rats are more sensitive to the stimulating actions of exogenously administered prolactin (Demarest and Moore, 1981) and the inhibitory actions of environmental stress (Lookingland et al., 1990) than are males.

5.2.2. Androgens

As noted above, TIDA neuronal activity in male rats increases following orchidectomy and this effect is reversed by testosterone replacement (Gunnet et al., 1986; Toney et al., 1991). Thus, testosterone (or its active metabolite dihydrotestosterone) inhibits the activity of TIDA neurons, suggesting that the lower basal activity of these neurons in the male versus the female is due, at least in part, to the presence of testicular androgens. The orchidectomy-induced activation of TIDA neurons is partially dependent upon the presence of circulating concentrations of prolactin since it is prevented by administration of the DA agonist bromocriptine (Toney et al., 1991). On the basis of these results it has been suggested that testosterone attenuates the responsiveness of TIDA neurons to the tonic stimulatory actions of prolactin, a conclusion consistent with the finding that TIDA neurons in gonadally-intact males are less sensitive than females to the tonic stimulatory actions of prolactin (Demarest and Moore, 1981). On the other hand, testosterone does not disrupt the ability of TIDA neurons to respond to exogenously administered prolactin (Toney et al., 1991).

DA neuronal input enhances the responsiveness of steroid primed neuronal pathways involved in male sexual behaviors including sexual motivation, and genital and motor responses. Evidence indicates that both the mesotelencephalic and the diencephalic DA neuronal systems are involved in these processes. For example, in the presence of a receptive female rat and during the act of copulation there is activation of nigrostriatal DA neurons for the initiation and execution of copulatory movements, mesolimbic DA neurons for sexual motivation/appetite, and PeVDA neurons projecting to the medial preoptic area (in males) and ventromedial nucleus (in females) for modulating sensory processing and integrating sexual behaviors. Pharmacological studies suggest that PeVDA neurons may integrate male copulatory behavior via an action on multiple DA subtypes in the medial preoptic area. Stimulation of D_1 receptors in the early phase of copulation activates the parasympathetic nervous system to cause erection, whereas stimulation of D_2 receptors during copulation activates the sympathetic nervous system to cause seminal emission and ejaculation. During both the precopulatory period (when males are exposed to a receptive female behind a barrier) and actual copulation, the concentrations of DA (collected via microdialysis from the medial preoptic area) are increased (Hull et al., 1997, 1999). These actions of PeVDA neurons are androgen-dependent since orchidectomy decreases precopulatory and copulatory behaviors (and the release of DA from medial preoptic area), whereas testosterone restores these behaviors and DA release in a temporally-related fashion (Putnam et al., 2001). On the basis of these results it has been proposed that testosterone serves as a permissive factor for DA release in the medial preoptic area in male rats exposed to receptive females (Hull et al., 1997, 1999; Putnam et al., 2001).

6. NEURONAL REGULATION OF DIENCEPHALIC DA NEURONS

Little information is available regarding the neuronal regulation of diencephalic DA neurons as compared with the mesotelencephalic DA neurons. Nonetheless, several neurotransmitter systems have been implicated in the neural control of these DA neurons based on (1) early anatomical studies showing colocalization of specific neurotransmitters and their receptors in regions of the hypothalamus containing perikarya or axon terminals

of DA neurons, and (2) recent pharmacological studies demonstrating that activation or blockade of these receptors alters either the basal activity of diencephalic DA neurons, or the response of these neurons to altered physiological or endocrinological conditions. The majority of these studies have focused on TIDA neurons (and to a lesser extent PHDA/THDA neurons terminating in the posterior pituitary) and identified both stimulatory and inhibitory neuronal systems important in the regulation of these DA neurons. The experimental evidence suggests that some neuronal systems act, at least in part, as mediators of hormonal feedback regulation of TIDA neurons, whereas others participate in suppression of the activity of these DA neurons during physiological states associated with episodic prolactin release. In addition, several of these neurotransmitters have been found to be colocalized in TIDA neurons suggesting autoregulatory and/or paracrine roles for coreleased neurotransmitters in the control of anterior pituitary hormone secretion. The finding that some colocalized neurotransmitters are only found in TIDA neurons under certain endocrine conditions suggests that circulating hormones may regulate expression and release of colocalized neurotransmitters from these neurons.

There are sexual differences in the responses of diencephalic DA neurons to a variety of pharmacological and physiological manipulations that may reflect differences in the neuronal systems regulating these DA neurons. For example, TIDA neurons in females are more responsive to the stimulating actions of prolactin (Moore, 1987a), the inhibitory effects of stress (Lookingland et al., 1990), and administration of kappa opioid agonists (Manzanares et al., 1992b) and the N-methyl-D-asparate (NMDA) receptor antagonist MK801 (Wagner et al., 1993). On the other hand, activation of the TIDA neurons after administration of bombesin (Toney et al., 1992) and a kappa opioid antagonist (Manzanares et al., 1992b) is more pronounced in males. Additional details of the responses of TIDA and other diencephalic DA neurons in male and female rats are provided in the following sections which describe the actions of individual drugs.

6.1. STIMULATORY NEUROTRANSMITTERS

Several neuropeptide and amino acid neurotransmitters are reported to stimulate TIDA neurons including neurotensin, bombesin-like peptides (acting at gastrin-releasing peptide [GRP] receptors) and glutamate (acting at both NMDA and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid [AMPA] receptors). By virtue of their ability to activate TIDA neurons these neurotransmitters suppress pituitary prolactin secretion, but many of these paradoxically increase prolactin release through direct actions on anterior pituitary lactotrophs. Only glutamate tonically stimulates TIDA neurons, but exclusively in females through an action on NMDA receptors. Selective pharmacological activation of delta opioid receptors also stimulates TIDA neurons suggesting that enkephalins may play a excitatory role in the neuronal regulation of TIDA neurons. Since enkephalin also has a prominent inhibitory influence on TIDA neurons these will be discussed along with other opioid neuropeptides in Section 6.2.

6.1.1. Neurotensin

Neurotensin is a tridecapeptide neurotransmitter implicated in the regulation of a variety of anterior pituitary hormones including prolactin, growth hormone, thyrotropin, adrenocorticotropin and the gonadotropins (for review, see Aronin et al., 1986; Rosténe

and Alexander, 1997). Control of anterior pituitary hormone secretion by neurotensin occurs via direct actions on the pituitary endocrine cells, as well as through regulation of the release of hypothalamic stimulatory and inhibitory neurosecretory neurons. Neurotensin is synthesized in neurons as part of a larger proneurotensin precursor protein which contains a single copy of peptide whose biological activity resides predominantly in the carboxyl terminus (i.e. residues 8–13; Granier et al., 1982; Dobner et al., 1987; Kislauskis et al., 1988). Like all peptide neurotransmitter precursors, proneurotensin is proteolytically cleaved during axonal vesicular transport such that under normal conditions immunoreactive neurotensin is found primarily in axon terminals and (to a lesser extent) dendritic processes, rather than in neuronal perikarya (Nicot et al., 1995a). In the colchicine-treated animals (where anterograde transport of neuropeptide is blocked) neurotensin synthesizing neurons are found to be distributed heterogenously throughout the brain (Carraway and Leeman, 1976; Uhl et al., 1976, 1977; Jennes et al., 1982) including regions of the hypothalamus containing diencephalic DA neurons (Kahn et al., 1980; Ibata et al., 1984a,b; Goedert et al., 1985).

There are two general types of neurotensin-containing neurons present within the hypothalamus, including the local intrinsic neurons and the hypophysiotropic neurosecretory neurons. Local intrinsic neurons act primarily as interneurons and have neurotensin-IR fibers and the neurotensin receptors located in the same regions as neurotensin-IR perikarya (Kahn et al., 1980; Merchenthaler and Lennard, 1991; Rosténe and Alexander, 1997). In the DM-ARC and periventricular nucleus, these neurons terminate in close proximity to TH-IR neurons suggesting that neurotensin participates in neuronal regulation of TIDA and PHDA neurons (Marcos et al., 1996a). Hypophysiotropic neurosecretory neurons project to the median eminence and posterior pituitary gland where neurotensin released from these neurons regulates hormone secretion via a direct pituitary site of action (Kahn et al., 1980; Jennes et al., 1984; Ibata et al., 1984a,b; Kiss et al., 1987). Approximately 70% of all neurotensin neurons projecting to the median eminence originate in the ARC, whereas the remaining 30% come from the parvocellular region of the paraventricular nucleus (Merchenthaler and Lennard, 1991), likely colocalized in CRH neurosecretory neurons (Sawchenko et al., 1984). Neurotensin released from these neurons could stimulate prolactin secretion during stress via a DA independent mechanism.

Neurotensin is also contained in some (but not all) TIDA neurons in the DM-ARC and in 'DOPAergic' neurons in the VL-ARC (Everitt et al., 1986), suggesting that neurotensin may modulate the activity or function of these neurons. On the other hand, neurotensin detected in these neurons could represent peptide that has been internalized as a result of binding to neurotensin membrane receptors (Beaudet et al., 1994; Laduron 1995; Rosténe and Alexander 1997). Retrograde axonal transport of ligand bound neurotensin receptors to perikarya is believed to act as a novel messenger system modulating gene expression in central DA neurons (Laduron, 1995). The finding that few TH-IR axon terminals in the median eminence contain neurotensin despite the close proximity of individual TH and neurotensin fibers (Fuxe et al., 1984; von Euler et al., 1990) is consistent with the hypothesis that neurotensin is sequestered and metabolized within TIDA neurons, rather than synthesized and released into the hypophysial portal circulation by these neurons. There are no reports showing colocalization of neurotensin mRNA and TH in neurons in the ARC.

Radioligand binding experiments reveal both high and low affinity binding sites for neurotensin in the mammalian brain (Mazella et al., 1983; Kitabgi et al., 1985; Vincent,

1995; Vincent et al., 1999). The high affinity neurotensin binding sites include NTR_1 (Mazella et al., 1985) and the newly classified sortilin-like NTR_3 receptors (Vincent et al., 1999; Mazella, 2001; Navarro et al., 2001). NTR_1 belongs to a superfamily of G-protein-coupled receptors linked to multiple signal transduction pathways including protein kinase C-dependent intracellular calcium mobilization (Berry and Gudelsky, 1992), formation of cAMP (Yamada et al., 1993), phosphatidyl inositol hydrolysis (Watson et al., 1992) and nitric oxide synthesis (Marsault and Frelin, 1992). NTR_3 receptors are not coupled to G-proteins and are believed to regulate cellular trafficking in targeted neurons (Vincent et al., 1999; Mazella, 2001; Navarro et al., 2001). Low affinity 'levacobastine-sensitive' neurotensin binding sites are classified as NTR_2 and belong to a distinct superfamily of G-protein-coupled receptors than NTR_1 receptors (Chalon et al., 1996). Both the NTR_1 and the NTR_2 receptors are found in the hypothalamus in moderate density (Rosténe and Alexander, 1997); the NTR_1 isoform is predominantly expressed by neurons, whereas the NTR_2 isoform is expressed by glial cells (Schotte et al., 1988). NTR_1 is found in high density on both neuronal perikarya and axon terminals of mesotelencephalic DA neurons (Dana et al., 1989; Boudin et al., 1996), and it is well established that neurotensin acts directly to regulate the activity of these DA neurons (Binder et al., 2001). In contrast, only low to moderate levels of NTR_1 mRNA are expressed by neurons the DM-ARC and adjacent periventricular nucleus (Nicot et al., 1994), and it is controversial as to whether TIDA neurons contain neurotensin receptors (Nicot et al., 1995b; Alexander, 1997).

Several lines of experimental evidence reveal that neurotensin activates TIDA neurons (and thereby suppresses prolactin secretion) via a central site of action. Indeed, icv administration of neurotensin stimulates the neurochemical activity of TIDA neurons associated with DA release in the median eminence (Gudelsky et al., 1989; Pan et al., 1992) and inhibits prolactin secretion (Maeda and Frohman, 1978; Vijayan and McCann, 1979; Koenig et al., 1982; von Euler et al., 1990; Pan et al., 1992); whereas systemic neurotensin administration increases prolactin secretion (Maeda and Frohman, 1978; Vijayan and McCann, 1979; Login et al., 1990), but has no effect on TIDA neurons (Fuxe et al., 1984). Neurotensin-induced activation of prolactin release is blocked in the presence of DA (Login et al., 1990) suggesting that under physiological conditions when both of these neurotransmitters are present at cognate receptors on lactotrophs, DA inhibition of prolactin secretion predominates. Presumably, neurotensin released from neurosecretory neurons in the median eminence is transported in the portal blood to the anterior pituitary where it stimulates prolactin secretion, whereas neurotensin released locally from intrinsic neurons in the ARC counteracts this by stimulating DA release from TIDA neurons. Neurotensin-induced activation of TIDA neurons occurs via a protein kinase C-dependent mechanism indicating an action at the NTR_1 subtype of the neurotensin receptor (Berry and Gudelsky, 1992).

Whether neurotensin acts directly at NTR_1 receptors on TIDA neurons is not clear. The presence of dense neurotensin binding sites located within the mediobasal hypothalamus (Goedert et al., 1985; Meister et al., 1989) and the observation that single unit activity of approximately 70% of all DM-ARC neurons is stimulated by neurotensin in vitro suggest a localized action on (or near) TIDA neurons (Lin and Pan, 1993). Moreover, the presence of synaptic connections between neurotensin-IR and TH-IR neurons in the DM-ARC is consistent with a direct action of neurotensin on TIDA neurons (Marcos et al., 1996a). On the other hand, TIDA neurons do not normally express appreciable amounts of neurotensin receptor mRNA like mesotelencephalic DA neurons

(Nicot et al., 1995b), and there is very little overlap between the distribution of mRNAs for TH and neurotensin receptors in the DM-ARC or adjacent periventricular nucleus (Nicot et al., 1995b). Collectively, these results suggest that the stimulatory actions of neurotensin on TIDA neurons is not due to a direct action per se, but rather to activation of excitatory interneurons within the ARC.

These are no sexual differences in the regulation of TIDA neurons by neurotensin (i.e. central administration of neurotensin stimulates the activity of TIDA neurons in both males and females; Gudelsky et al., 1989; Pan et al., 1992). Similarly, pharmacological blockade of NTR_1 receptors has no effect on TIDA neuronal activity in either gender suggesting that local intrinsic neurotensin neurons in the DM-ARC do not tonically stimulate TIDA neurons and are not involved in gonadal steroid-dependent sexual differences in the basal activity of these neurons (Hentschel et al., 1998). In contrast, blockade of NTR_1 receptors prevents prolactin-induced delayed activation of TIDA neurons in both males and females indicating that neurotensin mediates (at least in part) prolactin feedback regulation of these neurons (Hentschel et al., 1998). The finding that prolactin stimulates FRA expression in neurotensin-IR neurons (and increases the total number of neurotensin-IR neurons) in the DM-ARC at time points preceding activation of TIDA neurons is consistent with this conclusion (Hentschel et al., 1999).

Interestingly, expression of neurotensin mRNA in the DM-ARC and the amount of neurotensin-IR in the median eminence of ovariectomized females are both increased by estrogen, and neurotensin mRNA expression in the DM-ARC is higher in cycling females during proestrus as opposed to diestrus (Alexander, 1993). Furthermore, approximately 80% of all neurotensin-IR neurons in the DM-ARC have estrogen-inducible progesterone receptors (Alexander, 1999). These results suggest that ovarian steroids regulate synthesis and release of neurotensin from neurosecretory terminals in the median eminence, which in turn stimulates episodic prolactin secretion via a DA-independent mechanism (Rosténe and Alexander, 1997). Whether estrogen or progesterone regulate neurotensin release from intrinsic neurons in the ARC is not known. It is important to note that during lactation (when the activity of TIDA neurons is suppressed) there is a marked increase in the colocalization of neurotensin-IR in TH-IR fibers in the median eminence (Ciofi et al., 1993; Marcos et al., 1996b). While the physiological significance of this phenotypic change is not known, it may represent a functional transition in the neurochemical nature of TIDA neurons important for maintenance of suckling-induced prolactin secretion (Rosténe and Alexander, 1997).

There have been several studies characterizing the stimulatory effects of neurotensin on PHDA neurons terminating in the intermediate lobe of the posterior pituitary. Central (icv) administration of neurotensin increases the activity of PHDA neurons and this causes a concomitant decrease in concentration of αMSH in plasma (Pan et al., 1992). Neurotensin stimulates DA release in vitro from explants containing PHDA neurons, but not from isolated neurointermediate lobes, which is consistent with a hypothalamic site of action of neurotensin in stimulating the activity of PHDA neurons (Davis and Kilts 1987a,b). Neurotensin causes a sustained excitation of DA release from PHDA neurons with little or no tolerance as compared with short-lived transient responses of mesolimbic DA neurons to neurotensin (Davis and Kilts, 1987b).

Little information is available regarding the effects of neurotensin on diencephalic DA neurons comprising the IHDA or PeVDA systems. Central administration of neurotensin increases DOPAC concentrations in whole hypothalamus (Widerlöv et al., 1982; Miñano et al., 1988), but the contribution of activated TIDA neurons to tissue DOPAC levels

cannot be ruled out in these studies. High density NTR_1 binding sites have been found in the medial portion of the MZI and adjacent dorsomedial hypothalamic nucleus (Fuxe et al., 1984; Moyse et al., 1987; Nicot et al., 1994), suggesting that neurotensin may regulate IHDA neurons via an action on receptors located on their perikarya and/or dendritic processes (Chan-Palay et al., 1984; van den Pol et al., 1984). On the other hand, there is little overlap in the distribution of TH mRNA- and neurotensin receptor mRNA-containing cells in the zona incerta (Nicot et al., 1995), making it doubtful that IHDA neurons are directly responsive to neurotensin.

Moderate densities of neurotensin binding sites have also been found in the rostral periventricular nucleus (Nicot et al., 1994) suggesting that neurotensin may regulate PeVDA neurons located in this region. Central administration of neurotensin inhibits both growth hormone and thyrotropin secretion (Maeda and Frohman, 1978), and it has been postulated that this occurs through DA neuronal stimulation of periventricular somatostatin neurosecretory neurons (Frohman et al., 1982). Direct injection of neurotensin into the medial preoptic nucleus increases luteinizing hormone secretion (Ferris et al., 1984), and this effect is prevented by blockade of DA receptors (Akema and Kimura, 1989). These results suggest that PeVDA neurons may also mediate the stimulatory effects of neurotensin on GnRH neurons.

6.1.2. Bombesin and related peptides

Bombesin is a tetradecapeptide originally isolated from an amphibian skin (Anastasi et al., 1971) and found to have a wide range of biological activities in mammals (Walsh et al., 1979). Mammalian bombesin-like peptides constitute a family of peptide neurotransmitters which include gastrin-releasing peptide (GRP; and its terminal fragment neuromedin C) and neuromedin B (NMB; Steel et al., 1992; Spindel et al., 1993). Classification of bombesin-like peptides is based on differences in the amino acid sequences of their amidated carboxy-terminal octapeptide domains, regions critical for receptor binding and biological activity (Kroog et al., 1995). Functional studies have revealed that bombesin-like peptide neurotransmitters act within the brain to regulate gastric acid secretion, gastrointestinal motility, body temperature, circulating glucose levels, satiety, stereotypic behaviors, and pituitary hormone secretion (Battey and Wada, 1991). Bombesin-like immunoreactivity is heterogenously distributed throughout the brain with the highest densities found in discrete hindbrain, midbrain and hypothalamic areas including the ARC (Moody et al., 1981) and suprachiasmatic nucleus (van den Pol and Gorcs, 1986; Mikkelsen et al., 1991; Abrahamson and Moore, 2001). The differential distribution of the bombesin receptor subtypes in the brain (Ladenheim et al., 1992; Pinnock et al., 1994) suggests that separate populations of GRP and NMB neurons regulate these various physiological functions. In addition, bombesin-like IR cells are also located in the anterior and intermediate pituitary lobes suggesting that locally released bombesin-like peptides may participate in the regulation of pituitary hormone secretion (Steel et al., 1992; Houben et al., 1993).

High affinity bombesin peptide binding sites have been identified within the rodent brain that comprise two pharmacologically distinct receptor populations; GRP-preferring (GRP-R) and neuromedin-preferring (NMB-R) subtypes (Battey and Wada, 1991; Ladenheim et al., 1992; Kroog et al., 1995). Although there is some overlap in their distribution, GRP-R are predominantly expressed in the hypothalamus, whereas NMB-R are found in highest levels in the olfactory and central thalamic regions (Kroog et al.,

1995). GRP-R are differentially distributed in discrete hypothalamic regions including the suprachiasmatic nucleus, supraoptic nucleus, paraventricular nucleus, medial preoptic area and lateral mammillary nucleus (Battey and Wada, 1991; Pinnock et al., 1994). In the suprachiasmatic nucleus, both GRP and GRP-R mRNA are expressed in high levels and it is believed that GRP may be released locally to act at high affinity receptors in the regulation of circadian rhythmicity (Albers et al., 1991; Battey and Wada, 1991). In agreement, GRP and neuromedin C were found to be equally potent in stimulating the in vitro activity of neurons in the suprachiasmatic nucleus via an action at GRP-R (Pinnock et al., 1994). Bombesin-like peptide receptors are also present in the anterior pituitary gland, mainly in subpopulations of lactotrophs and somatotrophs suggesting a direct action of GRP and/or NMB to regulate prolactin and GH secretion (Houben et al., 1994; Morel et al., 1994). The findings that (1) mRNAs encoding both GRP and NMB, and their receptors are expressed in the anterior pituitary gland (Houben et al., 1993) and (2) administration of GRP stimulates GH and prolactin secretion in vitro (Morel et al., 1994) suggest that locally synthesized bombesin-like peptides could act in a paracrine fashion to regulate release of these hormones.

The distribution of GRP-IR fibers and axon terminals in discrete hypothalamic nuclei (and their absence from the median eminence; Kentroti et al., 1988) suggests that central GRP neurons also regulate anterior pituitary hormone secretion, but indirectly via alterations in the activity of hypothalamic neurosecretory neurons. In the case of GH, GRP and bombesin are potent inhibitors of both basal and GHRH stimulated GH secretion (Kentroti et al., 1988; Kentroti and McCann, 1996), whereas immunoneutralization of hypothalamic GRP increases GH secretion (Kentroti et al., 1988). These results suggest that GRP neurons tonically inhibit GH secretion, and it has been suggested that this occurs via a neuronal pathway involving DA receptor-mediated stimulation of somatostatin neurosecretory neurons (Kentroti and McCann, 1996). If this is the case, then PeVDA neurons (acting via D_1 receptors in the PeVN) could mediate the inhibitory effects of bombesin-like peptides on GH secretion by stimulating somatostatin neurons. Although GRP-IR fibers are reported to terminate in the PeVN (Kentroti et al., 1988), it is not known if bombesin-like peptides stimulate the activity of PeVDA neurons located in this region. On the other hand, bombesin-like peptides could inhibit GH secretion via a somatostatin-independent mechanism involving activation of DA release from TIDA neurons and consequent direct DA inhibition of GH secretion via inhibitory D_2 receptors on somatotrophs (Goldsmith et al., 1979). The finding that bombesin stimulates the activity of TIDA neurons (Manzanares et al., 1991a) is consistent with this hypothesis, but it is not known if bombesin causes sufficient DA release to overcome the relative insensitivity of somatotrophs to DA for this to be physiologically relevant.

The majority of experimental evidence supports the conclusion that bombesin-like peptides stimulate the activity of TIDA neurons and thereby inhibit pituitary prolactin secretion. Indeed, bombesin administration does not suppress α-methyltyrosine- or haloperidol-induced prolactin secretion suggesting that pharmacological impairment of TIDA neuronal function prevents the prolactin inhibitory actions of bombesin (Collu et al., 1983; Buydens et al., 1988). Moreover, bombesin-like peptides block both opiate- and stress-induced prolactin secretion (Tache et al., 1979; Matsushita et al., 1983; Buydens et al., 1988), prolactin secretory responses known to be due, at least in part, to suppression of the activity of TIDA neurons (Moore and Lookingland, 1995). Central administration of bombesin increases DA synthesis and metabolism in whole hypothalamus (Widerlöv

et al., 1984) and DA turnover in the median eminence (Fuxe et al., 1980). Bombesin-induced activation of TIDA neurons is marked (but relatively short-lasting) and is accompanied by a concomitant decrease in plasma concentrations of prolactin (Manzanares et al., 1991a). The effects of bombesin on TIDA neurons and prolactin secretion are mimicked by equimolar concentrations of GRP and blocked by a GRP-R selective antagonist connoting an action at GRP-R (Manzanares et al., 1994). Blockade of GRP receptors per se is without effect, indicating that under basal conditions TIDA neurons are not under tonic excitatory control by GRP (Manzanares et al., 1994). Bombesin-like peptides likely act within the mediobasal hypothalamus to activate TIDA neurons since both bombesin and GRP (and to a lesser extent NMB; Lin and Pan, 1994) stimulate DA release (Kabayama et al., 1986) and single unit firing rate of DM-ARC neurons in hypothalamic slices in vitro (Lin and Pan, 1993).

Bombesin activation of TIDA neurons is not mediated by feedback activation by prolactin, but since bombesin's effects are more pronounced in ovariectomized than gonadally-intact rats it appears that in females, estrogen suppresses the stimulatory actions of bombesin on these neurons (Toney et al., 1992). Dependent upon the dose employed, central bombesin administration either attenuates and delays (low dose), or totally abolishes (high dose) diurnal afternoon surges of prolactin in estrogen-primed ovariectomized rats (Mai and Pan, 1993). This likely occurs through a mechanism involving activation of TIDA neurons since bombesin-induced inhibition of prolactin secretion is prevented by prior administration of a DA antagonist (Mai and Pan, 1993). In rats with lesions of the suprachiasmatic nucleus, daily prolactin surges are disrupted and bombesin-induced inhibition of prolactin secretion is abolished. This suggests that bombesin-like peptide-containing neurons in the suprachiasmatic nucleus may participate in photoperiod-induced rhythms in prolactin secretion (Mai and Pan, 1995). In agreement, GRP neurons in the suprachiasmatic nucleus are known to play a major role in the circadian timing system that regulates phasic secretion of anterior pituitary hormone secretion (Albers et al., 1991). GRP (coadministered with neuropeptides proline-histidine-isoleucine and vasoactive intestinal polypeptide; VIP) mimics the phase-delaying effects of light on circadian rhythmicity (Albers et al., 1991). GRP neurons in the ventral (or core) portion of the suprachiasmatic nucleus receive afferent photoperiod input from the retina and lateral geniculate (van den Pol and Gorcs, 1986; Mikkelsen et al., 1991; Abrahamson and Moore, 2001). Fibers from these GRP neurons terminate locally within the ventral suprachiasmatic nucleus, but also send projections to the dorsal hypothalamic area, rostral PeVN (Mikkelsen et al., 1991) and possibly the ARC (Moody et al., 1981). Thus, GRP (in conjunction with VIP) may participate in a neuronal pathway originating in the suprachiasmatic nucleus that limits the extent of episodic prolactin release via photoperiod-cued reactivation of TIDA neurons (see Section 7.1).

As compared with TIDA neurons, little information is available regarding the bombesin-like peptide regulation of the remaining diencephalic DA neurons. Central bombesin and GRP administration stimulates the activity of PHDA neurons terminating in the intermediate lobe and thereby inhibits melanotroph secretion of αMSH (Manzanares et al., 1991a). Bombesin-like peptides have also been implicated in the control of several anterior pituitary hormones, but whether diencephalic DA neurons are involved in this process has not been studied. For example, pharmacological blockade of bombesin receptors suppresses basal luteinizing hormone (but not follicle-stimulating hormone) secretion, whereas systemic (iv) administration has no effect (Pinski

et al., 1993). The suppressive effect of bombesin receptor blockade on luteinizing hormone secretion can be prevented by icv administration of GRP suggesting a role for GRP-R in the tonic stimulation of GnRH release. GRP has also been implicated in the regulation of the HPA axis via a stimulatory action on hypothalamic CRH neurons (Garrido et al., 1998). The presence of GRP-positive neurons in the parvocellular region of the paraventricular nucleus (Kentroti et al., 1988) is consistent with this hypothesis. The zona incerta receives bombesin-like IR innervation from the locus coeruleus (Lechner et al., 1993), but whether IHDA (and/or PeVDA) neurons mediate the actions of bombesin-like peptides on gonadotropin and adrenocorticotropin secretion remains to be elucidated.

6.1.3. Excitatory amino acids

Glutamate and aspartate represent the major excitatory neurotransmitters in the central nervous system. Of these, glutamate is found in higher abundance within the hypothalamus (Watkins and Evans, 1981), and glutamatergic axon terminals are reported to make synaptic contact with neuroendocrine neurons in the ARC, periventricular, paraventricular and supraoptic nuclei (van den Pol et al., 1990; Decavel and van den Pol, 1992; van den Pol and Trombley, 1993). On the basis of results from pharmacological studies employing selective glutamatergic receptor agonists and antagonists, glutamate has been implicated in neuroendocrine regulation of anterior pituitary hormone secretion, including steroid-induced luteinizing hormone secretion and preovulatory surges of luteinizing hormone and prolactin (Brann and Mahesh, 1994; Brann 1995).

Glutamate receptors are divided into two distinct groups, ionotropic and metabotropic receptors (Ozawa et al., 1998). Ionotropic receptors are associated with cation specific ion channels and comprise three groups; NMDA, AMPA and kainate-preferring receptors. NMDA receptors are highly permeable to calcium ions, undergo voltage-dependent blockade in the presence of physiological concentrations of magnesium ions, and have slow gating kinetics. These receptors are found predominantly in the forebrain, but are also present in lower densities throughout the rest of the brain. AMPA receptor channels mediate fast excitatory transmission across glutamatergic synapses and are permeable to sodium and potassium (rather than calcium) ions (van den Pol et al., 1990). These receptors are distributed ubiquitously throughout the brain, although regional differences in densities exist with especially high levels found in the hippocampus and lower levels of AMPA binding sites in the diencephalon, midbrain and brainstem. AMPA receptors have been identified on most diencephalic DA neurons (Chen et al., 2001). Kainate-preferring receptors are highly permeable to calcium ions and are found in high abundance throughout the entire brain, frequently coexisting with AMPA receptors on the same neuron. The metabotropic receptors are coupled to G-proteins and regulate the production of intracellular messengers (Ozawa et al., 1998).

Excitatory amino acid neurotransmitters (presumably released from interneurons located within the hypothalamus; van den Pol et al., 1990) are reported to stimulate the activity of TIDA neurons. Glutamate acting at NMDA receptors tonically stimulates the basal activity of TIDA neurons in female, but not male rats (Wagner et al., 1993). This sexual difference in NMDA receptor-mediated regulation of TIDA neuronal activity is likely due to estrogen-induced stimulation of glutamate release by a prolactin-independent mechanism (Wagner et al., 1993). In both genders, endogenous excitatory amino acids acting at AMPA receptors tonically inhibit the basal activity of TIDA neurons (Wagner

et al., 1994a) by a mechanism involving $GABA_A$ receptors (Wagner et al., 1994b). Blockade of AMPA receptors also stimulates the activity of PHDA neurons terminating in the intermediate lobe of the posterior pituitary and decreases secretion suggesting that glutamate maintains the basal activity of these neurons (Wagner et al., 1994b).

6.2. INHIBITORY NEUROTRANSMITTERS

Several inhibitory neurotransmitters have been implicated in the neuronal regulation of diencephalic DA neurons including the endogenous opioid neuropeptides β-endorphin, enkephalin and dynorphin (acting at mu and kappa opioid receptors) and GABA (acting at $GABA_A$ and $GABA_B$ receptors). Endogenous opioid peptide regulation of TIDA neurons is multifaceted and differs from that of other diencephalic DA systems. For example, TIDA neurons are inhibited by β-endorphin acting at mu opioid receptors, whereas the activities of IHDA and PeVDA neurons are stimulated following pharmacological activation of mu receptors. β-Endorphin does not inhibit TIDA neurons under normal conditions in either gender indicating that sexual differences in the basal activity of these neurons is not mediated by mu opioids. But mu opioid receptor mediated inhibition of TIDA neurons is more pronounced in females than males, and prolactin surges that occur in females during suckling and stress are believed to be due, in part, to the permissive effects of β-endorphin-induced inhibition of TIDA neurons and loss of DA inhibition of hormone secretion. On the other hand, dynorphin acting at kappa opioid receptors tonically inhibits TIDA neurons in males (but not females) and this partially accounts for androgen-dependent sexual differences in the activity of these neurons. Enkephalins inhibit TIDA neurons via an action at mu opioid receptors, but they may also act at delta opioid receptors to stimulate these neurons and thereby inhibit the secretion of prolactin. GABAergic neurons tonically inhibit TIDA neurons in both males and females via an action at $GABA_A$ receptors, but may further suppress the activity of these neurons through $GABA_B$ receptors.

6.2.1. Mu opioids

β-Endorphin is the predominant endogenous mu opioid receptor agonist derived from the large precursor molecule POMC (Gramsch et al., 1980). There is a pronounced regional heterogeneity in the distribution of POMC-derived peptides in the brain suggesting that precursor processing (and relative opioid activity) varies in axons of individual neurons (Millington et al., 1984; Mezey et al., 1985). POMC-IR neurons located in the ARC send axon projections throughout the hypothalamus, with particularly dense innervation found in the medial preoptic area, periventricular nucleus, parvocellular subdivision of the paraventricular nucleus, and neural lobe of the posterior pituitary (Joseph et al., 1985; Mezey et al., 1985; Horvath et al., 1992a). In the median eminence, POMC-IR fibers are found predominantly in the lateral portion of the zona interna (rather than in the external zone where the portal capillary system is located) suggesting that β-endorphin released from these neurons acts predominantly within the mediobasal hypothalamus to regulate anterior pituitary hormone secretion (Ibata et al., 1985; Kiss et al., 1985). β-Endorphin-IR axon terminals make synaptic contact with TH-IR perikarya and dendrites in the periventricular nucleus, MZI and (to a lesser extent) VL-ARC (Horvath et al., 1992b) suggesting that β-endorphin may regulate the activity of these neurons. The presence of

mu opioid receptors in these regions is consistent with this hypothesis (Desjardins et al., 1990; Mansour et al., 1994; Mitchell et al., 1998).

Drugs acting as selective agonists or antagonists at the mu opioid receptors produce characteristic patterns of responses of different DA neurons. Morphine and a variety of mu opioid agonists increase the activity of the mesotelencephalic DA neurons terminating in the striatum and limbic forebrain regions, but inhibit TIDA neurons (Moore and Lookingland, 1995). Inhibition of TIDA neurons is responsible, at least in part, for increased circulating levels of prolactin caused by mu opioid agonists in both male and female rats (Haskins et al., 1981; Selmanoff and Gregerson, 1986; Kapoor and Willoughby, 1990; Janik et al., 1992; He et al., 1994). The inhibitory action of mu opioids on TIDA neurons is due to their ability to hyperpolarize these neurons by increasing potassium conductance (Loose and Kelly, 1990; Lin and Pan, 1995; Wagner et al., 1997).

Compelling experimental evidence suggests that endogenous mu opioids participate in the regulation of TIDA neurons during lactation. Indeed, selective pharmacological blockade of mu opioid receptors prevents suckling-induced suppression of TIDA neurons (Callahan et al., 1996; Arbogast and Voogt, 1998) and prolactin secretion (Selmanoff and Gregerson, 1986; Maumann and Rabii, 1991; Arbogast and Voogt, 1998). Immuno-neutralization of β-endorphin, met-enkephalin and leu-enkephalin suppresses suckling-induced prolactin secretion suggesting that multiple mu opioid peptidergic neurons may be involved in the neural regulation of TIDA neurons during lactation (Jaworski et al., 1997; Callahan et al., 2000). The observation that suckling increases Fos expression in POMC-IR neurons in the ARC is consistent with an involvement of localized intrinsic β-endorphin neurons in this process (Pape et al., 1996).

Agonists and antagonists of mu opioid receptors do not alter PHDA neuronal activity in the intermediate lobe (Moore and Lookingland, 1995), but mu agonists inhibit both the basal and stimulated activity of THDA neurons terminating in the neural lobe of the posterior pituitary (Lookingland and Moore, 1985; Garris and Ben-Jonathan, 1990; Racké et al., 1990). The activity of IHDA neurons is stimulated following acute administration of morphine by a mechanism involving mu opioid receptors (Tian et al., 1992; Moore and Lookingland, 1995). Since mu opioid receptors cause postsynaptic inhibitory responses, it is likely that stimulation of IHDA neurons results from disinhibition of an unidentified tonically-active inhibitory interneuron. The stimulatory effects of mu opioid receptor activation on IHDA neurons are not dependent upon the presence of serotonin neurons since neurotoxin-induced disruption of serotoninergic innervation to the hypothalamus does not alter the ability of morphine to stimulate the activity of IHDA neurons (Tian et al., 1992). PeVDA neurons in the rostral periventricular nucleus that project to the medial preoptic nucleus are also activated following acute administration of morphine by a mechanism involving mu opioid receptors. In this respect, IHDA and PeVDA neurons resemble mesotelencephalic DA neurons rather than TIDA or THDA neurons (Moore and Lookingland, 1995).

6.2.2. Kappa opioids

Endogenous dynorphins (e.g. dynorphin$_{1-8}$ and dynorphin$_{1-17}$) are peptidergic kappa opioid receptor agonists derived from the large precursor molecule prodynorphin (Civelli et al., 1985). Prodynorphin mRNA-containing perikarya are found throughout the hypothalamus in both densely packed and loosely arranged groups (Merchenthaler

et al., 1997). Dense compact groups of prodynorphin mRNA-containing perikarya are predominantly found in the neuropil surrounding the suprachiasmatic nucleus as well as clustered within the anterior periventricular, dorsomedial, supraoptic and paraventricular nuclei (Morris et al., 1986). Dynorphin-IR in the supraoptic and paraventricular nuclei is predominantly localized within magnocellular vasopressin neurosecretory neurons projecting to the neural lobe of the posterior pituitary (Meister et al., 1990). A subpopulation of magnocellular neurons in the paraventricular nucleus also express kappa opioid receptor mRNA (Mansour et al., 1994) suggesting that dynorphin may act in an autoregulatory fashion to suppress vasopressin secretion from the neural lobe. Loosely arranged populations of prodynorphin mRNA-containing perikarya are scattered throughout most of the remaining regions of the hypothalamus (Merchenthaler et al., 1997), and these neurons likely account for the high concentrations of dynorphin-like IR (presumably in axonal processes) found throughout the rostrocaudal extent of the hypothalamus (Zamir et al., 1983, 1984). The codistribution of dynorphinergic neurons (Merchenthaler et al., 1997) and kappa opioid receptor mRNA-expressing cells (Mansour et al., 1994) within hypothalamic regions containing diencephalic DA neurons suggests that endogenous kappa opioids may act locally to regulate the activity of these neurons. The finding that pro-dynorphin-IR axons make synaptic contact with approximately 60–70% of all TH-IR neurons in the ARC is consistent with a direct action of dynorphin on TIDA neurons (Fitzsimmons et al., 1992).

Kappa opioid receptor mRNA-expressing cells are widely distributed throughout the hypothalamus and (in most regions) are more prevalent than those containing mu or delta receptor mRNA (Mansour et al., 1994). Drugs that act at kappa opioid receptors influence the activity of diencephalic DA neurons, but unlike mu opioid agonists (which depending on the neuronal system can increase or decrease the activity of DA neurons), kappa agonists exert only inhibitory actions. The degree of inhibition is generally dependent upon the level of activity of these DA neurons at the time the kappa agonist is administered. For example, the kappa agonist U50,488 exerts only minimal inhibition of TIDA neurons unless these neurons are activated (Manzanares et al., 1991b). U50,488 reduces the elevated level of activity of TIDA neurons in female rats, but is without effect in males unless the latter animals are injected with prolactin or are orchidectomized in order to activate their TIDA neurons (Manzanares et al., 1992b). On the other hand, the selective kappa opioid receptor antagonist norbinaltorphimine increases TIDA neuronal activity in gonadally-intact male (but not female) rats suggesting that in males TIDA neurons are tonically inhibited by the endogenous kappa opioid dynorphin (Manzanares et al., 1992b; Durham et al., 1996). In agreement, icv administration of dynorphin antibodies increases the basal activity of TIDA neurons in male rats (Manzanares et al., 1992c). Estrogen acts in females via a prolactin-independent mechanism to suppress kappa opioid-receptor-mediated inhibition of TIDA neurons, possibly by decreasing the release of endogenous dynorphin (Manzanares et al., 1992b; Wagner et al., 1994c).

Agonists and antagonists of kappa opioid receptors decrease and increase, respectively, the activity of PHDA neurons terminating in the intermediate lobe of the posterior pituitary and cause reciprocal changes in circulating concentrations of αMSH (Manzanares et al., 1990b, 1991c). The ability of dynorphin antibodies to mimic the stimulatory effects of kappa opioid antagonists on PHDA neurons suggests that these neurons are inhibited tonically by an endogenous dynorphin-containing neuronal system (Manzanares et al., 1992c). Activation of kappa opioid receptors has no effect on the

activity of IHDA neurons (Tian et al., 1992). No information is available regarding the effects of kappa receptor activation or blockade on the activity of PeVDA neurons.

6.2.3. Delta opioids

Delta opioid receptors in the brain participate in a variety of physiological processes including regulation of pituitary hormone secretion. The delta opioid receptor is a member of a seven transmembrane G-protein family of neurotransmitter receptors negatively coupled to adenylyl cyclase (Quock et al., 1999). Pharmacological studies have revealed the presence of two subtypes of delta opioid receptors in mammalian brain; δ_1-opioid receptors which preferentially bind the delta opioid agonist DPDPE and δ_2-opioid receptors which bind [D-Ala^2, Glu^4] deltorphin (Vanderah et al., 1994). In addition to their independent actions, δ_2-opioid receptors have been shown to be involved in the modulation of mu opioid receptor responses (Porreca et al., 1992). The distribution of delta opioid receptors within the hypothalamus is highly confined, with the highest density of binding located in the dorsomedial and ventromedial nuclei (Temple and Zukin, 1987). Delta opioid receptor labeling is sparse throughout the rest of the hypothalamus, including regions containing diencephalic DA neurons (Desjardins et al., 1990; Gouarderes et al., 1993). Although derivatives of proenkephalin (i.e. leu-enkephalin, met-enkephalin) bind with highest affinity to delta opioid receptors and are generally considered to be the major endogenous delta opioids (Mansour et al., 1986), there is considerable cross-reactivity of endogenous opioids with opioid receptor subtypes such that enkephalins may also bind to mu opioid receptors (Davis et al., 1985) and β-endorphin may bind to delta opioid receptors (Shook et al., 1988).

There is a high level of correspondence between the distributions of delta opioid receptor mRNA-containing cells and delta opioid receptor binding in rat brain (Mansour et al., 1993). Delta opioid receptor mRNA-containing cells have limited distribution within the hypothalamus and expression levels are very low in most regions except for the dorsomedial portion of the ventromedial nucleus (Mansour et al., 1994). In the rostral hypothalamus only low levels of delta receptor mRNA can be detected in scattered cells in the medial preoptic area, whereas more caudally, delta opioid receptor mRNA is not detectable in the periventricular nucleus, ARC or median eminence (Mansour et al., 1994). In contrast, there is a mismatch in the hypothalamic localization of proenkephalin mRNA and delta opioid receptor mRNA and binding (except in the ventromedial nucleus). Indeed, several hypothalamic areas including the paraventricular, dorsomedial and ventromedial nuclei, and lateral hypothalamic area all express high levels of proenkephalin mRNA, yet only the ventromedial nucleus contains delta opioid binding sites, cells expressing delta receptor mRNA (Mansour et al., 1993, 1994), and a high density of leu-enkephalin-IR fibers (Elde et al., 1976). This limited distribution of delta opioid receptors in the ventromedial nucleus suggests that only discrete populations of enkephalinergic neurons act through these receptors, whereas the remaining hypothalamic enkephalinergic neurons act through mu receptors. Interestingly, only marginal delta opioid binding is found in regions of the hypothalamus containing enkephalin-IR perikarya (Simantov et al., 1977; Sar et al., 1978) suggesting a lack of somatodendritic delta opioid autoreceptors on these enkephalinergic neurons (Desjardins et al., 1990).

Compared with the rest of the brain, the hypothalamus is relatively rich in both leu-enkephalin and met-enkephalin, with moderate to high levels found in most regions containing diencephalic DA neurons (Zamir et al., 1985), however, there is no sexual

difference in the concentrations of leu-enkephalin in the hypothalamus (Tang and Man, 1991). Numerous small met-enkephalin-IR perikarya in the rostral hypothalamus reside within the medial preoptic and periventricular nuclei (Merchenthaler et al., 1986b), and these cells colocalize vasopressin in the parvocellular division of the paraventricular nucleus (Sakanaka et al., 1990b). More caudally, numerous scattered, intensely stained cells are present in the ARC, suprachiasmatic, ventromedial and dorsomedial nuclei (Tramu et al., 1981; Merchenthaler et al., 1986b). Enkephalin-IR neuronal processes are uniformly distributed throughout the entire hypothalamus including the median eminence, infundibulum and posterior pituitary (Tramu et al., 1981; Merchenthaler et al., 1986b).

A variety of chemically-identified neurons within the ARC receive both indirect (extrinsic) and direct (intrinsic) enkephalinergic neuronal input (Magoul et al., 1993) suggesting a role for enkephalin in the neuroendocrine regulation of pituitary hormone secretion. Enkephalin-IR perikarya in the bed nucleus of the stria terminalis, medial preoptic nucleus, periventricular nucleus and dorsomedial nucleus all provide extrinsic input to the rostral ARC, whereas intrinsic enkephalin neurons connect the rostral and caudal portions of the ARC (Magoul et al., 1993). Enkephalin-IR neurons innervate TH-IR neurons (perikarya and dendrites) in the DM-ARC (but not in the VL-ARC), β-endorphin neurons in the VL-ARC, and NPY neurons in the ventromedial ARC (Magoul et al., 1994). There are symmetrical synaptic connections between enkephalin axon terminals and POMC perikarya in the ARC (Zhang et al., 1987), and reciprocal synaptic associations with NPY neurons in the ventromedial ARC (Li et al., 1993).

There have been few studies on the responses of hypothalamic DA neurons to drugs that act at delta opioid receptors, but these drugs exert a pattern of effects that is different from that of drugs acting on mu or kappa opioid receptors (Manzanares et al., 1993). In male rats an icv injection of [D-Pen^2, D-Pen^5]enkephalin, an delta opioid receptor agonist, has no effect on nigrostriatal DA neurons, but increases the activity of TIDA neurons. These effects are blocked by naltrindole, a selective delta-opioid receptor antagonist, but this antagonist has no effect per se on TIDA neurons. Met-enkephalin inhibits the single-unit activity of ARC neurons in hypothalamic slices obtained from diestrus and ovariectomized female rats, but since inhibition of only a minority of cells was blocked by naltrindole it is likely that inhibition is mediated by mu (rather than delta) opioid receptors (Lin and Pan, 1995). Interestingly, TH-IR neurons located in both the DM-ARC and VL-ARC synthesize enkephalin during lactation as opposed to a lack of synthesis in male or cycling female rats (Merchenthaler, 1993; Ottinger et al., 1995). It has been postulated that enkephalin may stimulate prolactin secretion during lactation by reversing the inhibitory action of DA and thereby maintaining elevated prolactin secretion during the non-suckling periods of lactation. TH-IR neurons in the ARC of pregnant, pseudopregnant and aging rats also contain enkephalin-IR and proenkephalin mRNA suggesting a role for prolactin in the stimulation of enkephalin synthesis in TIDA neurons (Merchenthaler, 1994). Enkephalin synthesis in TH-IR neurons in the ARC is unaffected by ovariectomy indicating this occurs independently of ovarian steroids (Merchenthaler, 1994).

Agonists and antagonists of delta opioid receptors do not alter PHDA neuronal activity (Manzanares et al., 1993). No information is available regarding the effects of delta opioid receptor activation or blockade on the activities of IHDA or PeVDA neurons in the rostral periventricular nucleus and medial preoptic nucleus.

6.2.4. GABA

GABA is the most ubiquitous inhibitory neurotransmitter in the mammalian central nervous system. The majority of GABAergic neurons are interneurons with short axons that project locally; they play a major role in most neuronal circuits. There are, however, some long projection neurons. For example, GABAergic neurons that project from the striatum to substantia nigra inhibit nigrostriatal DA neurons, and neurosecretory GABAergic neurons that project to the median eminence (van den Pol, 1986; Kosaka et al., 1987) have been postulated to inhibit the release of prolactin from the anterior pituitary (Racagni et al., 1982).

GABA released from nerve terminals activates either ionotropic $GABA_A$ or metabotropic $GABA_B$ receptors located on target cell membranes. The more prevalent ionotropic $GABA_A$ receptors gate a chloride ionophore. When GABA activates this receptor/chloride channel complex it promptly increases the frequency of opening of the chloride channel and thereby hyperpolarizes post-synaptic neurons. Agonists of $GABA_A$ receptors include muscimol and isoguvacin; bicuculine is an antagonist. Benzodiazepines act on allosteric receptors on the $GABA_A$/chloride channel complex to increase the affinity of GABA for the $GABA_A$ receptor and thereby enhance the action of this inhibitory neurotransmitter. Metabotropic $GABA_B$ receptors couple to calcium and potassium channels through G protein-mediated second messenger systems. That is, activation of $GABA_B$ receptors increases potassium conductance and thereby mediates a slow inhibitory postsynaptic potential; by reducing calcium conductance on nerve terminals GABA inhibits calcium-mediated neurotransmitter release. Baclofen is an agonist, and phaclophen and 2-hydroxysaclofen are antagonists at $GABA_B$ receptors.

There is extensive codistribution of GABA neuronal perikarya and their processes in brain regions containing diencephalic DA neurons, and GABA-IR has been detected in many TH-IR neurons within the hypothalamus (Kosaka et al., 1987). GABA-IR/TH-IR perikarya are present in both subdivisions of the ARC suggesting that GABA is colocalized in TIDA neurons in the DM-ARC and in 'DOPAergic' neurons in the VL-ARC (Everitt et al., 1984; Meister and Hökfelt, 1988). Both light and electron microscopy studies have revealed that GABA and TH coexist in nerve endings of TIDA neurons in the median eminence (Schimchowitsch et al., 1991), suggesting that under certain physiological conditions these neurons may corelease DA and GABA into the hypophysial portal blood. In addition, GABA-IR boutons make synaptic contact with virtually all diencephalic DA neurons (i.e. A_{11} to A_{14}; van den Pol, 1986). This suggests that GABA exerts an inhibitory modulatory control over all diencephalic DA neurons, especially TIDA neurons. Results of electrophysiological studies support this suggestion.

Several investigators have used the hypothalamic slice preparations to record the electrical activities from ARC neurons in response to GABA or its agonists and antagonists, but in only a few of these studies have recordings been made from identified TIDA or PHDA neurons. In vitro application of GABA inhibits the firing of antidromically identified TIDA neurons in rats (Nishihara et al., 1983). Intracellular recordings made from slices of guinea pig hypothalamus revealed that baclofen produces a dose-related hyperpolarization of ARC TH-IR neurons (Wagner et al., 1997). Moreover, in vivo baclofen reduces the activity of TIDA neurons and increases plasma concentrations of prolactin; effects that are blocked by the $GABA_B$ antagonist 2-hydroxysaclofen (Wagner et al., 1997). On the other hand, systemic injection of a $GABA_A$ receptor

antagonist increases TIDA neuronal activity and decreases plasma concentrations of prolactin, effects that are prevented by the $GABA_A$ agonist isoguvacine (Wagner et al., 1994). This finding indicates that these neurons are tonically inhibited by GABA via an action at $GABA_A$ receptors (Wagner et al., 1994b). Lee and Pan (2001) reported similar results following icv injections of $GABA_A$ agonists and antagonists in ovariectomized, estrogen-treated female rats. Muscimol, but not baclofen, reduced TIDA neuronal activity and increase circulating concentrations of prolactin; these effects were blocked by bicuculline. Bicuculline also reduced the afternoon surge of prolactin in these animals suggesting that GABA, acting through $GABA_A$ receptors, modulates basal and diurnal changes in TIDA neuronal activity and prolactin secretion. The functional significance of GABA localized in nerve terminals in the median eminence is less well defined although the results of some studies suggest that GABA released from the median eminence can act on the anterior pituitary to inhibit the secretion of prolactin (Racagni et al., 1982).

GABA is also found in PHDA neurons that innervate the intermediate lobe of the posterior pituitary in a variety of species (Vuillez et al., 1987; Schimchowitsch et al., 1991). Both the $GABA_A$ and D_2 receptors are located on melanotrophs and both transmitters hyperpolarize these cells and inhibit release of the POMC-derived peptides β-endorphin and αMSH (Millington and Chronwall, 1989; Goudreau et al., 1992). The question arises as to why two inhibitory neurotransmitter systems are needed to regulate hormone release from intermediate lobe melanotrophs, and it has been suggested that $GABA_A$ receptors mediate rapid responses of short duration, whereas D_2 receptors mediate slow G-protein-linked long-lasting responses (Sands et al., 1998). There is also evidence that $GABA_B$ receptors are also involved with GABA-induced hyperpolarization of melanotrophs (Taraskevich and Douglas, 1990). In agreement, MacVicar and Pittman (1986) found that electrical stimulation of the pituitary stalk evoked fast initial inhibitory post-synaptic potentials in intermediate lobe melanotrophs that were blocked by bicuculline, whereas prolonged hyperpolarization was blocked by DA antagonists. Baclofen (but not isoguvacine) reduces PHDA neuronal activity and increases plasma concentration of αMSH; effects that are blocked by $GABA_B$ antagonists (Goudreau et al., 1994). On the other hand, neither $GABA_A$ nor $GABA_B$ antagonists affect PHDA neuronal activity or αMSH secretion. Thus, PHDA neurons are not tonically inhibited by GABAergic neurons, but $GABA_B$ antagonists do block the inhibitory effects of stress on the activity of these neurons.

The GABA-containing axon boutons make synaptic contact with TH-IR neurons in the MZI (van den Pol, 1986) suggesting that GABAergic neurons regulate the activity of IHDA neurons. There is, however, little overlap in the location of GABA neurons (in the lateral/ventral zona incerta) and TH-IR neurons (in the medial/rostral zona incerta) or their projection sites (Oertel et al.,1982) suggesting that (unlike other diencephalic DA neurons) GABA is not colocalized in IHDA neurons (Kolmac and Mitrofanis, 1999). Although the functional consequences of GABA regulation of IHDA neurons are not well characterized, these DA neurons are reported to stimulate gonadotrophin release (MacKenzie et al., 1988). GABAergic neurons in the zona incerta inhibit luteinizing hormone release in ovariectomized, estradiol plus progesterone-treated rats (Wilson et al., 1990), and it has been hypothesized that progesterone-induced activation of IHDA neurons and luteinizing hormone secretion results from removal of a tonic inhibitory action of GABA on the activity of these diencephalic DA neurons (Kalia et al., 1999).

7. ROLE OF DIENCEPHALIC DA NEURONS IN THE REGULATION OF PROLACTIN SECRETION UNDER VARIOUS PHYSIOLOGICAL STATES

Prolactin is most frequently associated with the stimulation of milk synthesis in the mammary gland during lactation, although this hormone has a number of other physiological and behavioral functions in mammals (for review, see Freeman et al., 2000). In non-pregnant, non-lactating rats prolactin secretion is under inhibitory control by TIDA and PHDA/THDA neurons, but control of secretion of this hormone changes on the afternoon of proestrus, and during pregnancy, lactation and stress. Details regarding the regulation of TIDA neurons and prolactin secretion during the estrous cycle (Moore, 1987b), pregnancy and lactation (Freeman et al., 2000; Grattan, 2001; Voogt et al., 2001) have been previously reviewed and will be only highlighted in the following sections. References to the primary papers cited in these sections can be found in these reviews.

There are sexual differences in the regulation of prolactin secretion that are reflected by differences in daily patterns of hormone release between male and female rats. In males, prolactin secretion is maintained at low basal levels throughout the day via tonic DA receptor-mediated inhibition of pituitary lactotrophs (Ben-Jonathan, 1985; Shieh and Pan, 1996). In the absence of DA inhibition, however, individual lactotrophs obtained from males secrete prolactin in a discordant intermittent fashion (as compared with continuous secretion in females) suggesting that gonadal steroids may program gender-specific patterns of prolactin release via a direct action in the pituitary (Castaño et al., 1994; Castaño and Frawley, 1995). In cycling females, prolactin secretion is low during estrus, diestrus and in the morning of proestrus, but increases over the course of proestrus until peak concentrations are attained in the late afternoon. This pattern in prolactin secretion is maintained under constant light and abolished by lesions of the suprachiasmatic nucleus suggesting that proestrus morning to afternoon variation in hormone release is endogenously generated and entrained by light (Pan, 1996; Freeman et al., 2000), possibly via an action mediated by melatonin secreted by the pineal gland (Fuxe et al., 1972; Shieh et al., 1997; Chu et al., 2000).

Patterns in prolactin secretion in females occur independent of ovarian steroids, but both estrogen and progesterone alter the magnitude and duration of prolactin surges. Indeed, prolactin secretion increases only slightly in the afternoon of ovariectomized females (Demaria et al., 2000b), is more pronounced in estrogen-treated ovariectomized rats (Mai et al., 1994; Yen and Pan, 1998; Demaria et al., 2000b), and is temporally advanced and enhanced in estrogen plus progesterone-treated ovariectomized rats (Yen and Pan, 1998; Demaria et al., 2000b). Prolactin surges in steroid-treated ovariectomized rats resemble that seen on the afternoon of proestrus (Pasqualini et al., 1988; DeMaria et al., 1998), and although prolactin release can occur in the absence of a change in diencephalic DA neuronal activity (e.g. during stress in males), steroid-induced prolactin surges are generally believed to involve loss of DA neuronal inhibition of prolactin release concurrent with stimulation of hormone secretion by hypothalamic prolactin releasing factors (Pan, 1996; Freeman et al., 2000). Patterns of prolactin secretion become more complex during pregnancy, displaying mating-induced nocturnal and diurnal surges. Regulation of these surges likely occurs through the interplay of multiple neuronal pathways that cause oscillatory inhibition of diencephalic DA neurons and stimulation of release of prolactin releasing factors distinctly associated with nocturnal or diurnal prolactin surges (Arey and Freeman, 1989; Freeman et al., 2000). Similarly, suckling- and

stress-induced prolactin surges have been associated with simultaneous suppression of diencephalic DA neuronal activity and activation of prolactin-releasing factor release. The role of DA in the generation of rhythmic prolactin release under various physiological states is discussed in the following sections.

7.1. PHOTOPERIOD

Episodic prolactin secretion in females is preceded by decreases in the activities of TIDA neurons in the median eminence (Fuxe et al., 1972; Mai et al., 1994; Shieh and Pan, 1996; Demaria et al., 2001b) and PHDA/THDA neurons in the posterior pituitary (Demaria et al., 2001b). The morning to afternoon diminution of TIDA neuronal activity develops during the peripubertal period in females (but not males; Shieh and Pan, 1998), and (like prolactin) its magnitude and timing is influenced by ovarian steroids (Mai et al., 1994; Shieh and Pan, 1996), and blocked by constant light (Fuxe et al., 1972; Shieh et al., 1997) and lesions of the suprachiasmatic nucleus (Mai et al., 1994). These changes in the neurochemical activity of diencephalic DA neurons associated with DA release from axon terminals in the afternoon are often accompanied by changes in gene expression in perikarya of these neurons. Indeed, expression of FRA in TH-IR neurons in the DM-ARC and periventricular nucleus decrease during the afternoon of proestrus which is consistent with a role for circadian variations in the activities of TIDA and PHDA/THDA neurons in mediating, at least in part, steroid-induced prolactin surges (Lerant and Freeman, 1997). In contrast, FRA expression in TH-IR neurons in the VL-ARC does not vary during proestrus connoting that 'DOPAergic' neurons in this region do not participate in the regulation of rhythmic prolactin secretion (Lerant and Freeman, 1997). Interestingly, TIDA and PHDA/THDA neuronal activities (Mai et al., 1994; Demaria et al., 2001b) and FRA expression (Lerant and Freeman, 1997) decrease in the afternoon in ovariectomized rats (in the absence of appreciable prolactin release) suggesting that lessening of DA inhibitory tone is insufficient to cause prolactin surges in the absence of ovarian steroids.

Although little information is available regarding the neuronal pathways that regulate diurnal changes in the activities of diencephalic DA neurons and prolactin secretion, the results from early tract-tracing and lesion studies suggest that the neurons originating in (or passing through) the suprachiasmatic nucleus may be involved. Indeed, TH-IR neurons in both the DM-ARC and the periventricular nucleus receive neuronal projections from the suprachiasmatic nucleus (Horvath, 1997), and bilateral lesions of this region prevent diurnal variations in the activity of TIDA neurons and prolactin secretion (Mai et al., 1994). Results from more recent neuroanatomical and gene expression studies suggest that VIP neurons may be involved in the generation of these rhythms; (1) VIP fibers terminate in close proximity to TH-IR perikarya and proximal dendrites in the DM-ARC and periventricular nucleus (Gerhold et al., 2001), (2) TIDA and PHDA/THDA neurons express VIP receptors (Gerhold et al., 2001), (3) diurnal variations in Fos expression in VIP neurons in the suprachiasmatic nucleus correlate with FRA expression in TIDA and PHDA/THDA neurons (Gerhold et al., 2002), and (4) direct administration of anti-VIP antisense into the suprachiasmatic nucleus prevents afternoon decreases in FRA expression in TIDA and PHDA/THDA neurons (Gerhold et al., 2002). It has been postulated that VIP neurons in the suprachiasmatic nucleus may relay time-of-day cues to neuroendocrine TIDA and PHDA/THDA neurons that inhibit their activities and initiate afternoon surges in prolactin secretion (Gerhold et al., 2002).

It should be noted, however, that VIP is reported to stimulate (rather than inhibit) the activity of TIDA neurons (Huang and Pan, 1996; Freeman et al., 2000).

On the basis of pharmacological studies, several other central neurotransmitters have been proposed to participate in neuronal regulation of rhythmic activity of TIDA neurons and prolactin secretion in estrogen-treated ovariectomized rats. These include prolactin-releasing factors [TRH (Wang et al., 1989) and oxytocin (Yuan and Pan, 1996)], serotonin (acting at $5HT_{2A}$ receptors; Liang and Pan, 2000), acetylcholine (acting at nicotinic receptors; Shieh and Pan, 1995, 1997; Chu et al., 2001), β-endorphin and dynorphin (acting at mu and kappa opiate receptors, respectively; Shieh and Pan, 1997; Chu et al., 2001), GABA (acting at $GABA_A$ receptors; Lee and Pan, 2001), and nitric oxide (Yen and Pan, 1999). Blockade of opiate receptors prevents nicotine-induced inhibition and diurnal afternoon suppression of TIDA neurons suggesting that acetylcholine may act to inhibit these DA neurons by stimulating the release of inhibitory opioid peptides (Shieh and Pan, 1997). This inhibitory cholinergic/opioidergic neuronal pathway likely acts in parallel (rather than in series) with inhibitory serotonergic neurons (Liang and Pan, 2000), since blockade of prostaglandin synthesis prevents both cholinergic and opiate receptor-mediated inhibition of TIDA neurons, but not that mediated by serotoninergic receptors (Chu et al., 2001).

In seasonal breeding species, such as the hamster, shortening the light phase of the daily photoperiod suppresses prolactin secretion that in males, but not females (Krajnak et al., 1994) is accompanied over the course of several weeks by a decrease in DA concentrations in the median eminence (Steger et al, 1984; Benson, 1987; Krajnak et al., 1994, 1995). This apparent loss of DA stores in axon terminals of TIDA neurons occurs with little change in DA synthesis or metabolism in the median eminence (Krajnak et al., 1994, 1995), and does not involve loss of TH-IR neurons in the ARC (Krajnak and Nunez, 1996). On the other hand, the numbers of DDC containing cells in the ARC are fewer in hamsters maintained in short versus long days raising the possibility that light exposure may alter the DA synthetic capacity of TIDA neurons via regulation of DDC expression (Krajnak and Nunez, 1996). The observation that appreciable amounts of DOPA are present in the median eminence of short-day exposed hamsters in the absence of pharmacological inhibition of DDC (Krajnak et al., 1994) is consistent with the hypothesis that during the non-breeding season TIDA neurons in males have a diminished capacity to convert DOPA to DA which results in diminution of DA stores in axon terminals in the median eminence (Krajnak and Nunez, 1996). Interestingly, pinealectomy prevents the inhibitory effect of short days on median eminence DA concentrations suggesting that melatonin may mediate the effects of photoperiod on TIDA neurons (Bartke and Steger, 1992).

Pharmacological blockade of DA receptors increases prolactin secretion in hamsters maintained under short day conditions (Badura and Goldman, 1992) suggesting that (despite their diminished capacity to synthesize DA) TIDA neurons are still capable of maintaining tonic DA inhibition of pituitary prolactin secretion. This may be due, in part, to an increased responsiveness of pituitary lactotrophs to DA in male hamsters with gonadal regression (Steger et al., 1985) and, in part, to increased DA release from PHDA/THDA neurons terminating in the posterior pituitary (Steger et al., 1995).

7.2. ESTROUS CYCLE

Circulating concentrations of prolactin are low and relatively constant throughout the 4–5 days of the estrous cycle in the female rat, but during the afternoon of proestrus there is a

preovulatory surge of prolactin and luteinizing hormone. The increased release of prolactin on the afternoon of proestrus appears to result from progressively increasing blood levels of estradiol, but the mechanisms by which the proestrous surge of prolactin occurs are not completely known. Immediately prior to the prolactin surge on the afternoon of proestrus, (and in other physiological situations where increases in circulating concentrations of prolactin occur during pregnancy, lactation, stress, see below), there is a reduction in DA receptor-mediated inhibition of lactotrophs in the anterior pituitary. This results from a decrease in TIDA neuronal activity; the portal blood concentration of DA, activity of TH, and turnover of DA in the median eminence decline on the afternoon of proestrus prior to the increased secretion of prolactin. A reduction in the activity of PHDA and THDA neuronal activity may also be involved. However, the magnitude of the release of prolactin during some of these physiological states cannot be explained solely by a reduction of inhibitory DA tone, suggesting that prolactin releasing factors such as TRH, VIP, oxytocin, and less well defined prolactin-releasing peptides are also involved. The release of pituitary lactotrophs from tonic DA inhibition prepares pituitary lactotrophs to respond more effectively to prolactin releasing factors. Thus, the increased release of prolactin from the anterior pituitary during a variety of physiological stimuli results from a concomitant decrease in inhibitory DA tone and the stimulatory actions of prolactin releasing factors.

Little information is available regarding the neuronal network underlying inhibition TIDA neurons during proestrus that facilitates episodic prolactin surges, but the finding that pharmacological blockade of mu opiate receptors prevents the proestrous surge of prolactin (Ieiri et al., 1980) suggests that β-endorphinergic neurons are involved.

7.3. PREGNANCY

During the first 10 days of pregnancy in the rat, there are two daily surges of prolactin (diurnal and nocturnal) which are required for maintenance of the corpus luteum and the secretion of progesterone. These surges, which are induced by cervical stimulation during mating, are associated temporally with twice daily reductions of TIDA neuronal activity (McKay et al., 1982; Arbogast and Voogt, 1991c). A similar pattern of TIDA and PHDA/THDA neuronal activity and serum concentrations of prolactin also occurs for 12 days in rats made pseudopregnant by artificial stimulation of the uterine cervix (McKay et al., 1982; Lerant et al., 1996). Thus, in these two situations when the serum prolactin concentrations are low, activities of these DA neuronal systems are high. β-endorphin neurons may also play a role in the prolactin surges (Voogt et al., 2001); mating increases expression of Fos protein in β-endorphin neurons in the ARC of female rats. Intravenous infusions of naloxone block mating-induced daily surges of prolactin during the first 10 days of pregnancy in the rat (Voogt et al., 2001).

Daily surges of prolactin in the rat cease by mid-gestation (day 10 of pregnancy) as lactogenic hormones released from the placenta maintain luteal function. These placental hormones also activate TIDA neurons and thereby inhibit the maternal secretion of prolactin from the anterior pituitary (Demarest et al., 1983a,b). Accordingly, during the last half of pregnancy serum prolactin levels remain low while TIDA neuronal activity is high (McKay et al., 1982). A similar pattern of prolactin concentrations occurs in mice and hamsters. β-endorphin neurons do not appear to play a role in the placental lactogen-induced stimulation of TIDA neurons and the subsequent decrease of prolactin secretion during the latter half of pregnancy in the rat. Placental lactogen concentrations in plasma

of non-human primates, cows and sheep also increase progressively during pregnancy, while pigs, rabbits, and dogs do not secrete placental lactogen. In these latter species, prolactin secretion remains high throughout gestation. In humans, both prolactin and placental lactogen increase progressively throughout pregnancy, suggesting that placental lactogen does not stimulate TIDA neurons to inhibit prolactin secretion. Thus, depending on the species, different strategies are employed to ensure the continued activation of PRL-Rs throughout pregnancy. Following parturition prolactin secretion from the anterior pituitary again becomes the source of lactogenic hormones in the maternal circulation.

On the last day of pregnancy in the rat, an extended nocturnal surge of prolactin again occurs, but this surge is not prevented by exogenous administration of prolactin or placental lactogen, suggesting that TIDA neurons have become insensitive to the stimulating actions of these hormones. Indeed, as is the case with prolactin surges during the first half of pregnancy, the antepartum prolactin surge is accompanied by decreased TIDA neuronal activity (Andrews et al., 2001) but the surge itself may be due, at least in part, to prolactin releasing factors. Nevertheless, a reduced responsiveness of TIDA neurons to prolactin is maintained during lactation (Voogt et al., 2001).

7.4. LACTATION

During lactation, adaptations occur in the mother which result in a reduced state of activity of TIDA neurons and thus provide for prolonged hyperprolactinemia required for mammogenesis and the continuous synthesis of milk. Suckling induces an acute increase in prolactin secretion from the anterior pituitary due, in part, to reflex activation of neuronal circuits which inhibit TIDA neurons (Moore, 1987; Freeman et al, 2000). This is reflected in suckling-induced decreases in DA turnover in the median eminence, DA concentrations in hypophysial portal blood, and mRNA expression, amount and activity of TH in the ARC (Grattan, 2001). These effects are promptly reversed if the pups are removed from the mother, resulting in the decreased circulating levels of prolactin.

Suckling-induced reduction of TIDA neuronal activity not only increases the release of prolactin by reducing the major inhibitory tone on the lactotrophs, but also increases the ability of these cells to respond to putative prolactin releasing factors. Suckling also reduces the activity of DA neurons terminating in the neural lobe of the pituitary; this causes an increased prolactin release from the anterior pituitary by decreasing amount of DA and increasing the amount of oxytocin delivered to the lactotrophs from the neurohypophysis (Crowley et al., 1987). Suckling does not alter the activity of nigrostriatal, mesolimbic or IHDA neurons (Selmanoff and Wise, 1981).

Continuous high concentrations of prolactin activate TIDA neurons in non-lactating rats but in the lactating dam, these neurons are less responsive to the actions of endogenous or exogenously administered prolactin. Thus, in the rat the stimulatory feedback of prolactin on TIDA neurons is suppressed during lactation (Demarest et al., 1983c) and this allows the suckling stimulus to increase the secretion of prolactin unopposed by inhibitory TIDA neurons. The mechanism by which this occurs is not completely understood, although it is not the result of a down-regulation of PRL-R in the brain. Endogenous opiates appear to be involved since in the lactating dam, enkephalin is expressed in TIDA neurons and this is associated with the down-regulation of TH expression (Merchenthaler, 1993). In addition, suckling increases the activity of β-endorphin neurons, which in turn, inhibit TIDA neuronal activity, although this latter

action was not observed consistently (Jaworski-Parman et al., 1997). As would be expected if endogenous opioids inhibit TIDA neurons in the suckling dam, naloxone infusions block the inhibitory actions of β-endorphin on TIDA neurons thereby increasing their activity and suppressing prolactin release (Voogt et al., 2001). Immunoneutralization of dynorphin, leu-enkephalin and met-enkephalin also prevents the suckling-induced inhibition of TIDA neuronal activity and prolactin secretion (Callahan et al., 2000). Thus, both opioid and TIDA (and possibly THDA) neurons play important roles in the regulation of prolactin secretion during lactation.

PRL-R are normally expressed in several brain regions of the diestrus female rats, and in some of these regions (e.g. choroid plexus, medial preoptic nucleus, ARC) expression of these receptors increases during pregnancy and lactation. Moreover, during lactation PRL-R are expressed in brain regions where they are not normally found (e.g. paraventricular, supraoptic and ventromedial nuclei). These changes in PRL-R expression may play a role in maternal behaviors and neuroendocrine adaptations that occur during pregnancy and lactation (Grattan, 2001). For example, binding sites for prolactin in the choroid plexus may be involved in the transport of this relatively large polypeptide hormone (197–199 amino acids) into the brain. The increased expression of PRL-R in choroid plexus of pregnant and lactating rats may be responsible for increased transport of prolactin into brain and its action there to induce and maintain maternal and feeding behaviors (Grattan, 2001). PRL-R may be involved with the establishment of appropriate behaviors necessary to feed and nurture pups, adjust to nutritional demands of milk production, and to maintain blood levels of hormones necessary for the synthesis and secretion of milk. It is not known if the activation or inhibition of diencephalic DA neurons plays any role in these prolactin-stimulated behaviors. It has been proposed, however, that the hyperprolactinemia and increased expression of PRL-R during lactation may contribute to the suppression of the stress-induced HPA axis response in the lactating animals (Neumann, 2001).

Neuropeptide Y (NPY) has been identified in nerve terminals throughout the hypothalamus, particularly in the mediobasal hypothalamus where its expression increases during lactation. NPY is not normally present in TIDA neurons but expression in these neurons is induced by incoming neural signals activated by suckling. The functional consequences of NPY in lactating rats are confusing since this peptide has been reported to both increase and decrease the secretion of prolactin. Results of in vitro studies suggest that NPY amplifies the inhibitory actions of DA on prolactin secretion from cultured anterior pituitary cells derived from lactating rats (Wang et al., 1996), while in vivo studies demonstrate that NPY suppresses TIDA neuronal activity in lactating rats which leads to prolactin release (Li et al., 1999).

7.5. STRESS

Stressful situations activate mesolimbic and mesocortical DA neurons (see references in Lookingland et al., 1991; Fleckenstein et al., 1994; Le Moal, 1995), but under some circumstances can decrease impulse traffic in TIDA and PHDA neurons. As a consequence of removing DA inhibitory tone provided by the latter neuronal systems, stressful manipulations release prolactin from the anterior lobe and αMSH from the intermediate lobe of the pituitary (Moore, 1987; Lookingland and Moore, 1995).

Prolactin secretion increases following exposure of animals to a variety of stressful situations such as novel environments, swimming, ether, restraint, social conflict, etc.

However, only rigorous stressful procedures, such as restraint or forced swimming reduce TIDA neuronal activity (Demarest et al., 1985b). This suggests the involvement of prolactin releasing factors in most forms of stress (Gala, 1990). Furthermore, although stress increases the circulating levels of prolactin in both male and cycling female rats, only in the female is this increase accompanied by reduced activity of TIDA neurons (Demarest et al., 1985c). Testosterone tonically inhibits TIDA neuronal activity and makes these neurons less responsive to stressful stimuli. For example, restraint decreases activity of TIDA neurons in orchidectomized rats but not in orchidectomized rats treated with testosterone (Toney et al., 1991). Thus, a release of prolactin from the anterior pituitary in response to stressful stimuli results primarily from an action of prolactin releasing factors, although with some types of stressful manipulations (e.g. restraint preceded by exposure to ether) inhibition of TIDA neurons in cycling female rats contributes, possibly in a permissive fashion, to the prolactin response. Interestingly, stress has little effect on prolactin secretion in lactating rats (Kahoe et al., 1991).

Results of pharmacological studies employing antagonists to various neurotransmitters reveal that neurons that transmit signals with endogenous kappa and/or mu opioids, serotonin, histamine and acetylcholine are involved to some extent with stress-induced inhibition of TIDA neurons and the consequent secretion of prolactin (Demarest et al., 1985b,c; Freeman et al., 2001).

Secretion of the POMC-derived peptides (β-endorphin; αMSH) is increased by activation of β-adrenergic receptors and decreased by activation of DA receptors on melanocytes in the intermediate lobe of the rat pituitary gland (Smelik et al., 1983). Stress-induced release of results from the activation of β-adrenergic receptors by epinephrine released from the adrenal medulla and by reduced activity of PHDA neurons that terminate in the intermediate lobe of the pituitary (Lindley et al., 1990b). Physical restraint reduces DOPA accumulation and DOPAC concentrations in the intermediate but not the neural lobe of the pituitary of both male and female rats (Lookingland et al., 1991). The lack of sexual differences in the stress-induced suppression of PHDA neuronal activity contrasts with that observed in anatomically-related TIDA neurons. Stress-induced reductions of PHDA neuronal activity are usually accompanied by increased concentrations of αMSH in plasma. Neurons that transmit information by releasing histamine, serotonin and GABA play a role in the response of PHDA neurons to stress. That is, drugs that inhibit histamine synthesis or block H_1 receptors (Fleckenstein et al., 1994), destroy serotonin neurons or block $5HT_2$ receptors (Goudreau et al., 1993), or block $GABA_B$ (but not $GABA_A$) receptors (Goudreau et al., 1994) attenuate stress-induced inhibition of PHDA neurons.

In summary, during periods of some forms of stress TIDA and PHDA neurons receive a convergence of inhibitory inputs mediated, at least in part, by opioid, cholinergic, histaminergic, serotoninergic and GABAergic neurons. The inhibition of TIDA neurons (in female rats) and PHDA neurons (in both sexes) culminates in hormonal responses: the release of prolactin from lactotrophs in the anterior lobe of the pituitary and of αMSH from melanotrophs in the intermediate lobe of the pituitary.

8. SUMMARY AND CONCLUSIONS

The primary aim of this chapter is to present an overview of diencephalic DA neurons and their role in the neuroendocrine regulation of pituitary hormone secretion. Special

emphasis has been placed on the neuroanatomical distribution of these DA neurons in the hypothalamus and adjoining subthalamus, and the identification of hormonal feedback and neuronal pathways important in the regulation of these neurons during various physiological states. The most familiar diencephalic DA neurons comprise the TIDA system. These neurons lack true synapses and are neurosecretory in nature. DA released from their terminals in the median eminence diffuses through fenestrated capillaries of the hypophysial portal system and is transported to the anterior pituitary. Accordingly, TIDA neurons differ from the ascending mesotelencephalic DA neurons in that they lack presynaptic inhibitory DA autoreceptors and high affinity DA transporters. These neurons are uniquely regulated by opposing actions of DA on stimulatory D_2 and inhibitory D_1 receptors.

The primary function of DA released from TIDA neurons is to suppress prolactin secretion by acting on D_2 receptors on anterior pituitary lactotrophs. These neurons are also believed to mediate hyperprolactinemia-induced suppression of luteinizing hormone and GH secretion. TIDA neurons are tonically activated by prolactin and exhibit sexual differences in their basal activity. This results from a combination of the stimulatory effect of estrogen on prolactin secretion and a greater sensitivity to prolactin activation in females, and a prolactin-independent inhibitory effect of androgens in males. Prolonged elevations of prolactin induce changes in gene expression in TIDA neurons that result in delayed activation of these neurons in both females and males. Several neuronal systems within the hypothalamus have been implicated in mediating, at least in part, both tonic and induced prolactin feedback activation of TIDA neurons. Glutamatergic neurons tonically stimulate TIDA neurons exclusively in females through an action on NMDA receptors. Conversely, GABAergic neurons tonically inhibit TIDA neurons in both females and males via an action at $GABA_A$ receptors. TIDA neurons in males are also tonically inhibited by dynorphin, whereas in females estrogen acts to suppress kappa opioid inhibition of TIDA neurons. Stimulatory neurotensinergic neurons are not involved in sexual differences in the basal activity of TIDA neurons, but may mediate prolactin-induced delayed activation of DA release. On the other hand, stimulatory GRP neurons likely participate in a neuronal pathway that limits the extent of episodic prolactin release via photoperiod-cued activation of TIDA neurons. Neuronal systems within the hypothalamus also participate in suppression of TIDA neurons during physiological states associated with episodic prolactin release. Prolactin surges that occur in females during suckling and stress are likely due to the permissive effects of β-endorphin- and/or enkephalin-induced inhibition of TIDA neurons and a loss of DA inhibition of hormone secretion.

The PHDA neurons in the periventricular nucleus terminate in close proximity to intermediate lobe melanotrophs, and DA released from these neurons tonically inhibits secretion of αMSH and other POMC-derived peptides. PHDA neurons are regulated by DA autoreceptors, whereas THDA neurons terminating in the neural lobe are not. Neurotensin and GRP increase the activity of PHDA neurons and cause a concomitant decrease in concentration of αMSH in plasma. Agonists and antagonists of kappa opioid receptors decrease and increase, respectively, the activity of PHDA neurons, whereas neither mu or delta opioid receptors participate in the regulation of these neurons. The PHDA neurons are not tonically inhibited by GABAergic neurons, but $GABA_B$ antagonists block the inhibitory effects of stress on these neurons. Stressful manipulations release αMSH from pituitary melanotrophs by the combination of actions of epinephrine

released from the adrenal medulla and removal of DA inhibitory tone provided by the PHDA neurons.

The IHDA neurons in the MZI are likely involved in processing afferent 'sensory' information and integrating efferent neuroendocrine responses. IHDA neurons are regulated by inhibitory DA autoreceptors, and in this respect resemble DA neurons comprising the mesotelencephalic neuronal systems. IHDA neurons terminating in the paraventricular nucleus may regulate vasopressin and oxytocin release via an action on perikarya of magnocellular neurons. IHDA neurons may also participate in the regulation of CRH neurons in the parvocellular paraventricular nucleus. IHDA neurons are stimulated following pharmacological activation of mu receptors possibly by disinhibition of an unidentified tonically-active inhibitory interneuron. IHDA neurons may also be important in stimulating luteinizing hormone surges and ovulation via an action on GnRH neurons in the horizontal diagonal band of Broca. Progesterone-induced activation of IHDA neurons and luteinizing hormone secretion has been postulated to result from removal of a tonic inhibitory action of GABA on the activity of these diencephalic DA neurons.

The distribution of PeVDA neurons throughout the rostrocaudal extent of the periventricular nucleus suggests that the subpopulations of these neurons subserve different functions. PeVDA neurons in the rostroventral periventricular nucleus are components of a gonadal steroid responsive sexually dimorphic forebrain circuitry believed to mediate preovulatory gonadotropin secretion in females and copulatory behavior in males. The presence of DA receptors in the 'osmosensitive' anteroventral third ventricular region of the preoptic area suggests a stimulatory role for PeVDA neurons osmotic stimulation of vasopressin secretion. Based on their anatomical location it is likely that PeVDA neurons are involved in the DA receptor-mediated regulation of somatostatin neurons in the periventricular nucleus. PeVDA neurons in the dorsal periventricular nucleus project laterally into the adjacent parvocellular paraventricular nucleus and, in turn, receive axosomatic synapses from CRH neurons suggesting that these neurons may participate in the regulation of CRH neurosecretory neurons. PeVDA neurons are inhibited by DA autoreceptors and stimulated following activation of mu opioid receptors, but little else is known regarding neuronal regulation of these neurons.

9. ABBREVIATIONS

AP-1	activating protein-1
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
ARC	arcuate nucleus
CRH	corticotropin releasing hormone
COMT	catechol-O-methyltransferase
DA	dopamine; dopaminergic
DDC	DOPA decarboxylase
DM-ARC	dorsomedial arcuate nucleus
DOPA	3,4-dihydroxyphenylalanine
DOPAC	3,4-dihydroxyphenylacetic acid
FRA	Fos and its related antigens
GABA	γ-aminobutyric acid
GH	growth hormone

GHRH	GH releasing hormone
GnRH	gonadotropin releasing hormone
GRP	gastrin-releasing peptide
GRP-R	GRP-preferring neurotensin receptor
HPA	hypothalamic-pituitary-adrenal
HVA	homovanillic acid
IHDA	incertohypothalamic DA neurons
IR	immunoreactive
iv	intracerebroventricular
MAO	monoamine oxidase
MHPG	3-methoxy-4-hydroxyphenylethylene glycol
αMSH	α-melanocyte stimulating hormone
MZI	medial zona incerta
3MT	3-methoxytyramine
NMB	neuromedin B
NMB-R	neuromedin-preferring neurotensin receptor
NMDA	N-methyl-D-asparate
NPY	neuropeptide Y
NSD 1015	3-hydroxybenzylhydrazine
NTR	neurotensin receptor (NTR_1 NTR_2 NTR_3)
PeVDA	periventricular DA neurons
PHDA	periventricular-hypophysial DA neurons
POMC	proopiomelanocortin
PRL-KO	prolactin gene knockout
PRL-R	prolactin receptors
TH	tyrosine hydroxylase
TI	tuberoinfundibular
TRH	thyrotropin releasing hormone
VIP	vasoactive intestinal polypeptide
VL-ARC	ventrolateral arcuate nucleus

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CHAPTER IX

Human forebrain dopamine systems: Characterization of the normal brain and in relation to psychiatric disorders

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ABSTRACT

Impairments of dopaminergic neuronal populations have been strongly implicated in a variety of neurological, psychiatric and drug addiction disorders, mental illnesses. Although most research efforts have been directed toward understanding the dopamine neural system in animals, technological advances over the past two decades have also helped to expand our current knowledge about the discrete anatomical organization of the dopamine system in the human brain. Dopamine neuronal populations have now been characterized in the human brain from the level of gene transcription (using in situ hybridization histochemistry) to the distribution of related proteins (immunohistochemistry) and their receptors (as visualized in vivo by neuroimaging techniques of positron emission tomography (PET) and single photon emission tomography (SPECT). The insights revealed about the human dopaminergic system by these technologies should help in the development of more targeted treatment interventions of neuropsychiatric disorders. In this chapter, we have summarized the current status regarding the neuroanatomical organization of the human dopamine systems with focus on the forebrain. The general organization of the dopamine system, from the level of synthesis, release, uptake and receptors, in the human brain are described with their potential implications for addiction and psychiatric disorders.

1. INTRODUCTION

The dopamine system is intricately involved in a wide variety of neural systems that mediate motor control, emotional regulation, reward, motivation, cognition and endocrine function. Impairments of dopaminergic neuronal populations have been strongly implicated in a variety of neurological, psychiatric and drug addiction disorders, mental illnesses that have had a detrimental impact not only on the afflicted individual, but also on society. Although most research efforts have been directed towards understanding the dopamine neural system in animals, technological advances over the past two decades have also helped to expand our current knowledge about the discrete anatomical organization of the dopamine system in the human brain. Dopamine neuronal

populations have now been characterized in the human brain from the level of gene transcription (using in situ hybridization histochemistry) to the distribution of related proteins (immunohistochemistry) and their receptors (as visualized in vivo by neuroimaging techniques of positron emission tomography (PET) and single photon emission tomography (SPECT) as well as by post mortem immunohistochemistry and in vitro autoradiography). The insights revealed about the human dopaminergic system by these technologies should provide an enhanced possibility to develop better targeted strategies for treatment interventions of specific neuropsychiatric and addiction disorders. In this chapter, we will summarize the current status of knowledge about the anatomical organization of the human dopamine systems with focus on the forebrain. The general organization of the dopamine system from the level of synthesis, release, uptake and receptors in the human brain will be described with only limited reference to the animal literature (in particular primates which are most homologous to humans). The implications of the dopaminergic system for the wide variety of neuropsychiatric illnesses are too vast for the contents of this chapter, so only a brief review will be given for a few of these diseases, in particular addiction and psychiatric disorders.

2. ANATOMICAL ORGANIZATION OF DOPAMINE SYSTEMS IN THE NORMAL HUMAN BRAIN

2.1. DOPAMINE SYNTHESIS/DOPAMINE NEURONS

Dopamine is synthesized from the amino acid tyrosine by the enzymes tyrosine hydroxylase (TH; which forms L-3,4-dihydroxylphenylalanine, L-DOPA) and L-amino acid decarboxylase (AADC) in dopaminergic neurons. The mRNA expression of the TH (which is the rate-limiting enzyme in the synthesis of dopamine) is abundant throughout the human mesencephalon (Fig. 1); TH is a phenotypic marker for all catecholamines, dopamine, noradrenaline and adrenaline. Evidence for the presence of TH has been documented in the mesencephalon from at least 4.5 to 7 weeks of human fetal life

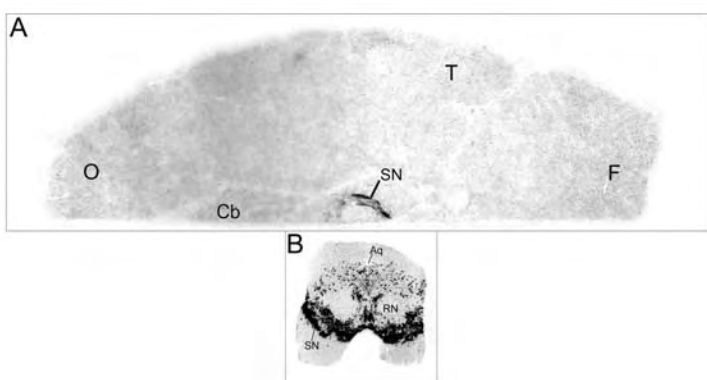

Fig. 1. Tyrosine hydroxylase mRNA expression in a whole hemisphere horizontal human brain section (A) and coronal section of the human mesencephalon (B). Note the lack of TH mRNA expression in the forebrain regions, but widespread expression throughout the mesencephalon.

(Freeman et al., 1991; Verney et al., 1991; Zecevic and Verney, 1995). The distribution of the TH cell groups at this developmental stage corresponds to the well known mesencephalic and hypothalamic dopaminergic groups (Zecevic and Verney, 1995). However, it is not until about week 19 in the human fetus that the TH-immunoreactive mesencephalic neurons have a similar organization to the adult with distinct delineation into the substantia nigra pars compacta, ventral tegmental area and retrorubral area (Aubert et al., 1997).

There is only one human TH gene, but there is an alternative splicing of the gene resulting in at least four types of the TH mRNA (Grima et al., 1987; Kaneda et al., 1987; Kobayashi et al., 1988; Le Bourdelles et al., 1988). At the protein level, the diversity is restricted to the N-terminal regulatory domain of the enzymes which suggests a means of regulating TH activity and thus dopamine synthesis. The four types of the TH isoform have been identified in terminal fields of the human brain (Lewis et al., 1993; Haycock, 2002). In contrast to the humans, the rodent and nonhuman primates have either no (only type 1) or two (types 1 and 2) alternative splicing variants in the brain, respectively. Studies of the TH gene in the brains of many species indicate that the multiplicity of the TH mRNA may be specific to primates (see Nagatsu, 1991, 1995; Haycock, 2002). TH mRNA types 1 and 2 dominate in the human brain with type 3 (barely detectable) and type 4 making up only about 0.5% of the TH mRNA population (Coker et al., 1990); see (Nagatsu, 1991). Both types 1 and 2 are coexpressed in the same ventral mesencephalic dopamine neurons (primarily containing neuromelanin) (Dumas et al., 1992; Lewis et al., 1994). The precise functional role of each isoform has not been determined, but the ratio of the TH mRNA type 1/type 2 appears to be larger in development than in the aged brain and the type 1 TH mRNA decreases with increasing age in adulthood (see Nagatsu, 1991). To date, however, no functional relevance has been documented in regard to the alternative splice variants of the TH mRNA.

The most abundant dopamine neurons (showing the highest expression of the TH mRNA) are located within the mesencephalic substantia nigra and ventral tegmental area that constitute the classic 'nigrostriatal' and 'mesocorticolimbic' pathways, respectively, as originally described in rodents (Dahlström and Fuxe, 1964; Björklund and Lindvall, 1984) though the topographical arrangement in primates is not as distinct. The organization of the mesencephalic dopamine neuronal populations have more recently been characterized into a 'dorsal' and 'ventral' tier cell group based on chemical characteristics and connectivity. Ventral tier dopamine neurons have a dorsoventral orientation consisting of the densocellular zone, ventrolateral substantia nigra 'pars lateralis', and cell columns of the substantia nigra pars compacta that radiates deep within the substantia nigra pars reticulata (Lynd-Balta and Haber, 1994a) and are characterized by low calcium binding protein (calbindinin-D28K) and the highest levels of TH mRNA expression (Haber et al., 1995; Fig. 2). The dorsal tier dopamine neurons have a mediolateral orientation and are located within the ventral tegmental area (consisting of the paranigral and parabrachial pigmented nuclei; homolog of the A10 region in rodents), retrorubral group (homologous to the rodent A8 rodent) and the dorsal part of the substantia nigra pars compacta (Poirier et al., 1983; Francois et al., 1985; Halliday and Törk, 1986; Lynd-Balta and Haber, 1994b; Haber et al., 1995) (Fig. 2). The dorsal tier neurons are characterized by positive calbindin binding (Lynd-Balta and Haber, 1994b; McRitchie et al., 1996) with relatively low TH mRNA expression as compared to the ventral tier (Haber et al., 1995). The connectivity of the dorsal and ventral tier neurons are described in Section 2.2. Although the expression of TH is higher in ventral tier neurons, there are a greater number

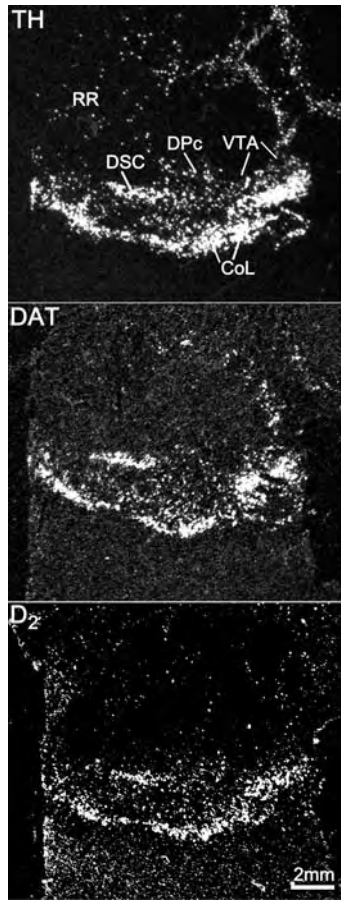

Fig. 2. Tyrosine hydroxylase (TH), dopamine transporter (DAT), and dopamine D_2 mRNA expression in the human mesencephalon. The dorsal tier cell group (dorsal part of the substantia nigra pars compacta, ventral tegmental area (paranigral and parabrachial pigmented nuclei), and retrobural area) are characterized by low mRNA expression levels of TH, DAT and D_2, whereas the ventral tier (ventral part of the substantia nigra pars compacta that includes the densocellular region dorsal to the pars reticulata and the cell columns that extend into the reticulata) is characterized by high mRNA expression levels of these dopaminergic markers.

of cells, presumably primarily dopaminergic, within the dorsal tier neuronal population (Halliday and Törk, 1986).

In addition to the mesencephalon, putative dopaminergic neurons have been documented in various human forebrain structures. Of these, the highest abundance is localized within the hypothalamus. Small hypothalamic dopaminergic neurons (immunoreactive for both TH and AADC) are distributed adjacent to the ependymal lining of the wall and ventral portion of the third ventricle corresponding to the arcuate nucleus-periventricular zone homologous with the A12, and A14 rodent cell groups (Kitahama et al., 1998). There are also medium- to large-sized dopaminergic cells throughout the posterior (extending from the central grey along the caudal wall of the third ventricle) and dorsomedial hypothalamic nuclei (A11) as well as in the caudal dorsal hypothalamic area (A13) (Kitahama et al., 1998). TH-immunoreactive neurons have also been identified in

the human basal forebrain substantia innominata that is considered to extend from the olfactory bulb homologous with the A16 cell group (Gaspar et al., 1985). Apparent dopaminergic neurons have also been detected in the human cerebral cortex, especially layer VI (Gaspar et al., 1987), and the subcortical white matter (Pearson et al., 1990). Scattered TH-immunoreactive neurons have also been identified in the human (Cossette et al., 1999; Prensa et al., 2000) and monkey (Betarbet et al., 1997; Betarbet and Greenamyre, 1999) striatum. The fact that these striatal neurons (GABAergic interneurons in appearance) all express the DA transporter strongly suggest that they are dopaminergic cells (Betarbet et al., 1997; Betarbet and Greenamyre, 1999).

2.2. DOPAMINE PATHWAYS

Dopaminergic pathways within the human forebrain are presented in Fig. 3. Immunological techniques have been mainly used to map the distribution of TH terminals in the human brain, and which show widespread innervation throughout the forebrain (McGeer et al., 1971; Pearson et al., 1979; Torack and Morris, 1990; Akil and Lewis, 1994a; Kung et al., 1998; Hedreen, 1999; Sutoo et al., 2001) (Fig. 4). The striatal complex is the most studied of the forebrain structures innervated by the mesencephalic dopamine cell groups; distinct mesencephalic dopamine pathways terminate in distinct striatal subregions of primates (Szabo, 1980; Francois et al., 1984; Haber and Fudge, 1997). Dopamine terminals reach the anlage of the human striatum already by gestational weeks 6 to 8 (Pearson et al., 1980; Freeman et al., 1991; Verney et al., 1991; Silani et al., 1994; Zecevic and Verney, 1995). Analysis of TH-immunoreactive nerve terminals in the human brain and anatomical tracing experiments in nonhuman primates has provided insights into the anatomical organization of putative dopamine neuronal pathways. The striatal dopaminergic targets are primarily the medium spiny projection neurons, which make up 70–80% of the striatal neuronal population (Graveland et al., 1985); the vast majority of the dopaminergic innervation is to the dendritic shafts with lower amounts to the spines and sparse innervation to the perikarya (Smith et al., 1994). Ultrastructural studies reveal that the dopaminergic innervation is anatomically situated to modulate cortical innervation to the striatum, which forms asymmetric synapses almost exclusively with the heads of dendritic spines (Smith et al., 1994). The dopaminergic innervation to the striatum has a ventral to dorsal increasing gradient that is mapped onto a gradient of limbic to associative to motor cortical input (see, e.g. Parent and Hazrati, 1995; Haber and Fudge, 1997). Through a series of ascending spiral feed-forward loops with the dopamine mesencephalic neuronal populations, limbic information is able to influence motor output since the ventral striatal cells terminate onto mesencephalic dopaminergic cells which in turn will innervate more dorsally localized striatal neurons (Haber et al., 2000; Fig. 5). This gradient emphasizes the fact that there lacks a distinct anatomical border between the dorsal and the ventral striatum, especially in humans.

The most dense dopaminergic innervation from the 'ventral tier' dopamine cell group is to the central (associative) and dorsolateral (sensorimotor) striatal regions. The dorsal tier does not project to the sensorimotor-related striatum. A wide expanse of the densocellular ventral tier cell group innervates the central striatum, with the majority arising from the densocellular cells, but the dorsal tier cell group of the substantia nigra also provides some innervation to the associative striatum. The dorsolateral striatum receives its innervation only from the ventral tier neurons (Haber et al., 2000). The more caudal dorsolateral striatal region receives its predominant dopaminergic innervation only from the cell

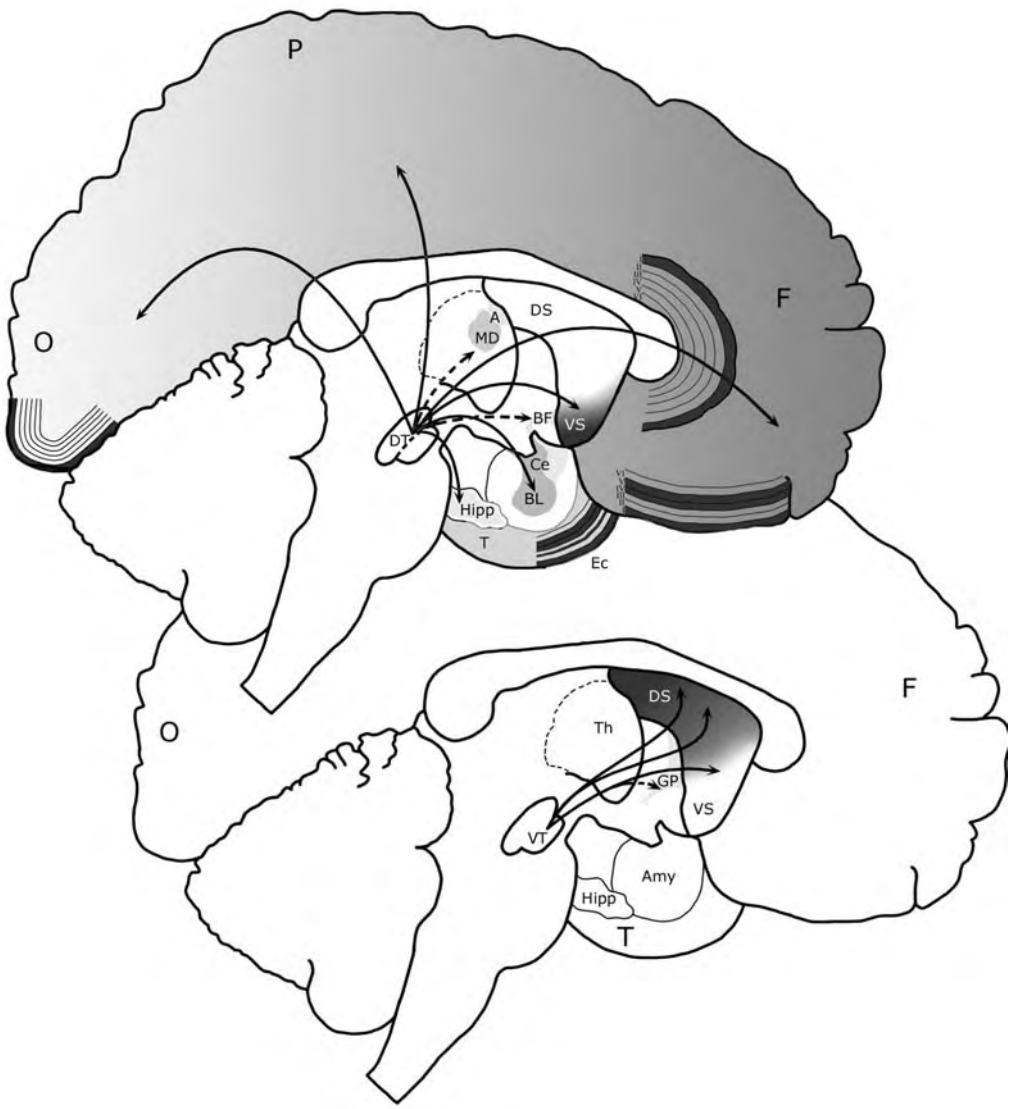

Fig. 3. Schematic illustration of proposed dopamine pathways in the human forebrain originating from the dorsal tier (DT) and ventral tier (VT) mesencephalic cell populations. A, anterior thalamus; BF, basal forebrain; BL, basolateral amygdala; Ce, central amygdala; DS, dorsal striatum (includes the caudate nucleus and putamen); Ec, entorhinal cortex; F, frontal cortex; Hipp, hippocampus; MD, mediodorsal thalamus; O, occipital cortex; P, parietal cortex; T, temporal cortex; VS, ventral striatum.

columns. The mesencephalic dopamine input to the ventral striatum arises predominantly from the 'dorsal tier' cell group. The nucleus accumbens region within the ventral striatum has been subdivided into a core and shell division in the rodent with the shell more linked to limbic function and the core associated with motor circuits (see Heimer et al., 1991; Zahm and Brog, 1992). A shell/core neurochemical distinction has also been observed in the nucleus accumbens of human (Voorn et al., 1994; Meredith et al., 1996) and monkey

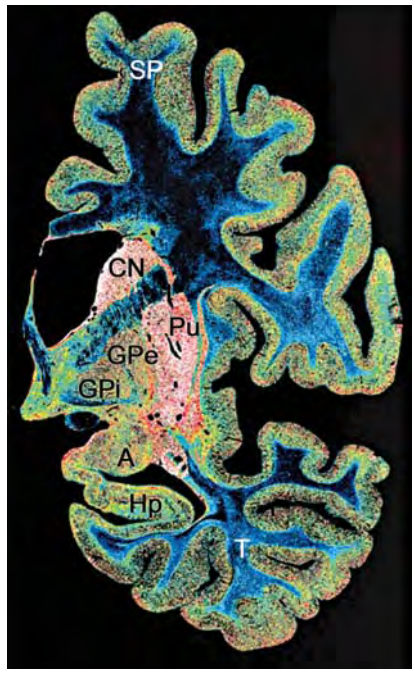

Fig. 4. Tyrosine hydroxylase immunoreactivity (TH-IR) in the human brain at the level of the posterior striatum (taken from Sutoo et al., 2001). Note highest TH-IR in the caudate (CN) and putamen (Pu), but significant levels are also evident in other brain regions such as the globus pallidus (external GPe and internal GPi), cerebral cortex temporal (T); superior frontal (SF), hippocampus (Hipp) and amygdala (Amy).

(Ikemoto et al., 1995; Meredith et al., 1996). The shell subregion of the primate nucleus accumbens is innervated almost exclusively by the ventral tegmental area of the dorsal tier mesencephalic cell group (Haber et al., 2000; Fig. 5). The core of the nucleus accumbens as well as the adjacent rostral ventral putamen and ventromedial caudate nucleus also receives innervation from the 'dorsal tier' as well as input from the densocellular part of the ventral tier mesencephalic neurons.

In addition to the ventral component of the striatum, there is a complex organization of the dorsal striatal region. The rostrocaudal extent of the dorsal striatum is distinguished into two compartments, patch (or striosome) and matrix (extrastriosome) regions (see Gerfen, 1992; Graybiel and Penney, 1999). Neurochemically, the patch/striosome compartment is characterized by low TH-immunoreactivity, low calbindin binding protein and low acetylcholinesterase activity. The patch/striosome compartment is preferentially innervated by limbic-related regions such as the amygdala (documented in the primate, rat, cat) and hippocampus (documented in the cat) as well as cortical innervation primarily from deep layer V neurons which are more abundant in periallocortical than neocortical areas (see Gerfen, 1992; Graybiel and Penney, 1999). In contrast, the matrix compartment in the human striatum is characterized by high TH-immunoreactivity, high acetylcholinesterase activity, and high calbindin binding and receives its cortical innervation primarily from sensorimotor regions. The dopaminergic innervation to the patch and matrix compartments of primates is not as clear as in rodents. Specific neuronal groups of dopaminergic neurons appear to differentially innervate the

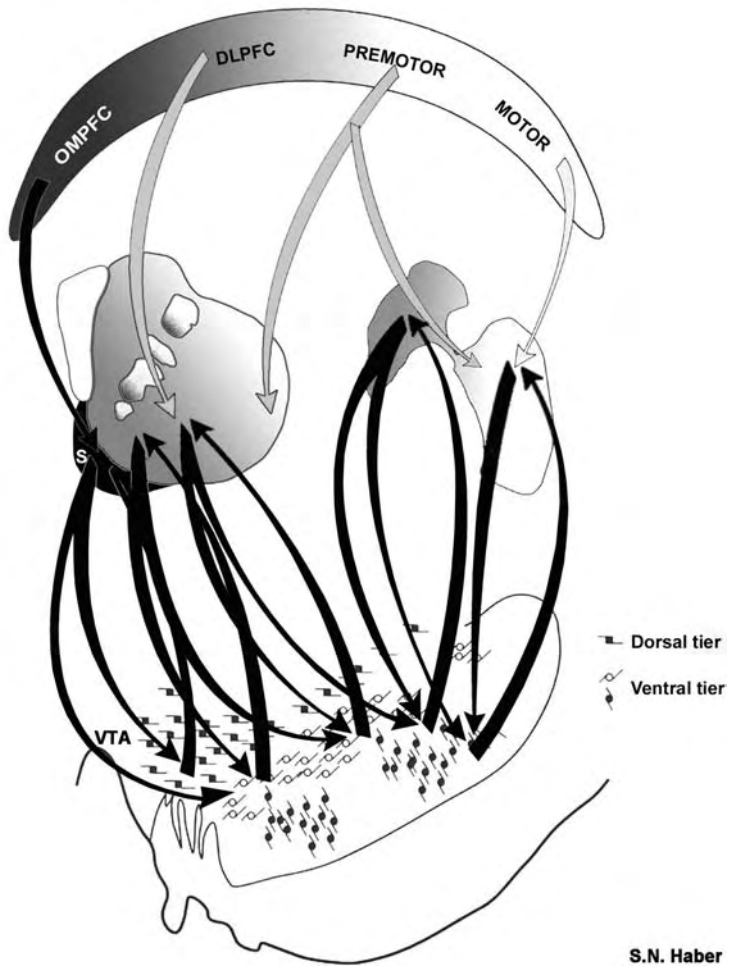

Fig. 5. Schematic illustration proposed by Haber et al. (2000) for the striatonigrostriatal spiral loop projection of the dorsal and ventral tier dopamine cells to the striatum. The gray scale gradient in rostral and caudal striatum illustrates the organization of functional corticostriatal inputs. Midbrain projections from the VTA to the nucleus accumbens shell form a 'closed', reciprocal striatonigrostriatal loop. Projections from the medial SN feed-forward to the nucleus accumbens core (immediately adjacent to the shell) forming the first part of a spiral. The spiral continues through the SNS projections with spiral pathways originating in the core and projecting more dorsally. As such, ventral striatal regions influence more dorsal striatal regions via spiraling SNS projections. DLPFC, Dorsolateral prefrontal cortex; OMPFC, orbital and medial prefrontal cortex; S, nucleus accumbens shell; VTA, ventral tegmental area.

striosome or matrix (see Graybiel and Penney, 1999). Neurons from the dorsal tier cell group as well as densocellular neurons appear to project preferentially to the matrix, whereas the ventral tier neurons innervate to a greater extent the striosome compartment (particularly within the caudate nucleus) (Langer and Graybiel, 1989). However, it has also been documented that the dorsal tier neurons do not project to the dorsolateral striatum in the monkey (Haber et al., 2000). The dorsolateral matrix-targeted dopaminergic cells may arise from the densocellular ventral tier group, adjacent to the dorsolateral ventral tier (see Haber and Fudge, 1997), but further examination is necessary

in the primate before the discrete anatomical organization of the dopaminergic cell groups to the patch/matrix organization is fully understood.

Axon collaterals of the 'ventral tier' neurons have been found to provide a sparse network of fine DA terminal fibers to the human globus pallidum (internal and external segments) as well as to the subthalamic nucleus (Lavoie et al., 1989; Parent and Lavoie, 1993a; Cossette et al., 1999; Augood et al., 2000); similar documentation in the monkey (Francois et al., 2000). However, there does not appear to be a specific pathway for these dopamine fibers. Instead, the fibers ascend along the major output pathways of the globus pallidus, in particular the ansa lenticularis and the lenticular fasciculus (see Prensa et al., 2000). In contrast to the striatum, the TH-immunoreactive varicosities in the globus pallidus are devoid of typical synaptic junctional apposition (Parent and Lavoie, 1993b) which suggests primarily a volume transmission means of communication for dopamine in this region.

In addition to the ventral striatum, the 'dorsal tier' mesencephalic neurons also project to the amygdaloid complex, basal forebrain and various cerebral cortical regions (Porrino and Goldman-Rakic, 1982; Gaspar et al., 1992; Lynd-Balta and Haber, 1994a). TH-immunoreactive axons that are positive for calbindin reach the anlage of the amygdaloid complex at 10.5 gestational weeks (Zecevic and Verney, 1995) and the first evidence of TH-immunoreactive fiber extension into the cortical anlage is evident at 7–8 gestational weeks with penetration into the cortical plate at 13 weeks of life (Zecevic and Verney, 1995). In the amygdaloid complex, apparent dopaminergic innervation is predominantly to the central (primarily the medial subdivision) and basolateral nuclei (Norita and Kawamura, 1980; Sadikot and Parent, 1990; Freedman and Shi, 2001). There is also apparent weak labeling within the accessory basal (basomedial) and corticomedial nuclei (Sadikot and Parent, 1990). The extended amygdala, which constitutes the extension of cell groups from the centromedial amygdala along the sublenticular substantia innominata to (and including) the bed nucleus of stria terminalis (see Heimer et al., 1997), also receives dopaminergic innervation from the dorsal tier neuronal dopaminergic population (Russchen et al., 1985; Freedman and Shi, 2001). There is a reciprocal innervation back to the dorsal tier mesencephalic cell group, but this arises primarily from the most lateral subdivisions of the bed nucleus of stria terminalis and central amygdala nucleus (Fudge and Haber, 2000, 2001). The presumable dopaminergic innervation to the primate substantia innominata includes the ventral pallidum (Gaspar et al., 1985; Lavoie et al., 1989); there is some evidence that this innervation arises in part from the ventral tegmental area (Irle and Markowitsch, 1986).

The dopaminergic pathway from the 'dorsal tier' neurons to the cerebral cortex is more expansive in primates than in rodents (see Berger et al., 1991). The most dense cerebral cortical innervation common to all species is to the prefrontal, anterior cingulate, insula and entorhinal cortices (for the primate: Porrino and Goldman-Rakic, 1982; Lewis et al., 1988b; Gaspar et al., 1992; Oeth and Lewis, 1992; Williams and Goldman-Rakic, 1993). However, in primates there is a widespread dopaminergic input throughout the cortical mantle including the motor, premotor and supplemental motor areas which receive strong innervation; the parietal, temporal and posterior cingulate receives a lighter innervation; the visual area receives very weak innervation (Campbell et al., 1987; Lewis et al., 1987, 1988a). In addition there is regional specificity with regard to the laminar distribution, depending on the particular cortical region. All cortical areas have apparent dopaminergic innervation to layer I. However, for example, there is dense innervation to layers I and V–VI in the frontal cortex, but there is a trilaminar organization (I, IIIa and V–VI) in the

entorhinal cortex (Lewis et al., 1988b; Akil and Lewis, 1993). Despite the lamination differences within the various cortices, there appears to be similar postsynaptic structural targets of the dopaminergic innervations in the different cortices. The majority of neocortical structures apposed to DA terminals are dendritic spines and shafts of excitatory pyramidal cells and a minority are associated with dendrites of the local inhibitory interneuron circuits; a similar pattern is observed in the entorhinal cortex. (Goldman-Rakic et al., 1989; Smiley and Goldman-Rakic, 1993; Sesack et al., 1995; Erickson et al., 2000). The fact that dopamine innervation is to layer V–VI pyramidal cells, which constitute the corticostriatal pathway, indicates that mesencephalic dopamine neurons can have both direct and indirect (via the cortex) modulation of striatal function. It is known that distinct mesencephalic neurons differentially innervate the cerebral cortex even though there is a partial intermingling of the dorsal tier cells with collateral axons to different cortical regions (Gaspar et al., 1992). Most studies have focused on the mesencephalic dopaminergic innervation to the frontal cortex (Gaspar et al., 1992; Williams and Goldman-Rakic, 1998). Experimental nonhuman primate studies have revealed that there is a topographic organization of the dorsal tier innervation to the frontal cortex. For example, the dorsal prefrontal cortex (Brodmann areas 46, 8B/6M and 4) receives dopaminergic input primarily from the entire medial to lateral extent of the dorsal cell group of the substantia nigra, the retrorubral area and a weaker innervation from the parabrachial pigmented nucleus (Williams and Goldman-Rakic, 1998). In contrast, the ventromedial prefrontal prelimbic and infralimbic cortices are innervated primarily from the ventral tegmental area, whereas the anterior cingulate is innervated by dopamine neurons located in the more medial extent of the dorsal cell group of the substantia nigra and retrorubral area. The ventral tier mesencephalic cell group does not appear to project to the cerebral cortex (Gaspar et al., 1992; Williams and Goldman-Rakic, 1998).

Dopaminergic innervation to the hippocampus archicortex is much weaker than that to the neocortex. However, similar to the other areas of cortical innervation, dopaminergic projections to the hippocampal formation of primates are also more widely distributed and dense as compared to the rodent (Gaspar et al., 1989; Samson et al., 1990; Akil and Lewis, 1994b). In addition to the hilus of the dentate gyrus, apparent dopaminergic innervation is evident in the CA region (stratum lacunosum moleculare) and the molecular layer of the subiculum (Samson et al., 1990). In contrast, the dopaminergic innervation in the rodent is primarily restricted to the ventral subiculum and to the adjacent CA1 region with very limited input to the rest of the hippocampal region (Gasbarri et al., 1994). In addition to direct hippocampal projections, there is a complex dopamine innervation of the entorhinal cortex (Akil and Lewis, 1993), which provides the major input to the hippocampus via the perforant pathway.

The presence of dopaminergic innervation is also evident in the dorsal thalamus of primates (Brown et al., 1979; Oke and Adams, 1987). Dopaminergic terminal thalamic innervation appears to be primarily within the mediodorsal, ventral anterior and anterior medial nuclei (Melchitzky and Lewis, 2001). These thalamic nuclei are tightly linked with limbic circuitry. The anterior nuclei, an important component of Papez's circuit, is involved in the transfer of information from the mammillary body to the cingulate gyrus. The mediodorsal thalamus receives its predominant innervation from the amygdaloid complex and the ventral striatopallidal system subsequently transferring information primarily to the prefrontal cortex including the orbitofrontal region. Though nigrothalamic projections have been documented for a long while in primates (Carpenter et al.,

1976; Ilinsky et al., 1985; Russchen et al., 1987), the specific origin of the dopaminergic cell groups which specifically innervate the thalamus are still undefined.

Overall, as predicted by anatomical organization and connectivity, the 'ventral tier' nigrostriatal dopaminergic system is primarily associated with basal ganglia function related to motor behavior, whereas the 'dorsal tier' mesocorticolimbic system is more linked to the neural structures involved in reinforcement, motivation, emotion and cognitive function.

2.3. DOPAMINE RECEPTORS

Dopamine exerts its various effects in the brain through activation of at least five different dopamine receptor subtypes – D_1, D_2, D_3, D_4 and D_5 (see Jackson and Westlind-Danielsson, 1994; Seeman et al., 1994; Jaber et al., 1996; Missale et al., 1998). The receptor subtypes are grouped into two major families, D_1-like and D_2-like, based on positive coupling to adenylate cyclase (D_1, D_5) and negative or uncoupling to adenylate cyclase (D_2, D_3, D_4). In the human fetus, D_1 DA mRNA transcripts, protein and binding sites are present at gestational week 12 and D_2 receptors are also transcribed in the striatum at that time (Brana et al., 1996). Very limited information is known, however, about the D_3, D_4 and D_5 receptor subtypes during human neurodevelopment, but in rats the D_3 receptor gene is present in the forebrain from at least gestational day 13 (Cadoret et al., 1993), comparable with the 5th (5.3–5.7) week of human development. Animal studies (Jung and Bennett Jr, 1996) have documented functional coupling of D_1 and D_2 DA receptors to their respective G-proteins at postnatal day 5 (comparable to week 25 of human development). Figure 6 shows the striatal mRNA expression of the D_1, D_2 and D_3 mRNAs at a midgestational stage of the human fetus development.

2.3.1. Dopamine D_1 receptor mRNA expression

In the normal adult human brain, the cells expressing the D_1 mRNA are localized predominantly within the striatum, cerebral cortex and bed nucleus of the stria terminalis (Hurd et al., 2001). There is extremely low or no appreciable detection of D_1 mRNA expression in the hippocampus, diencephalon, brainstem, or the cerebellum indicating that neurons situated in those regions have no major role in mediating D_1 receptor regulated functions in marked contrast to the multitude of D_2 and D_3 receptor mRNA-expressing neurons in discrete populations in some of these brain areas (see Sections 2.3.3 and 2.3.5).

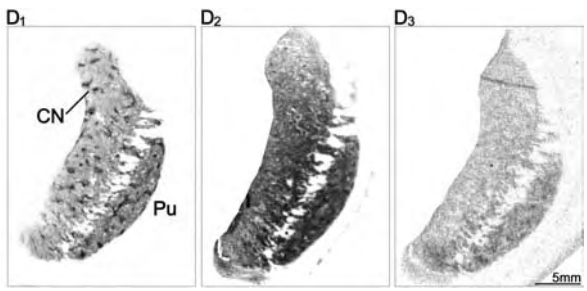

Fig. 6. The pattern of dopamine D_1, D_2, and D_3 mRNA expression in the rostral striatum of the human fetus (week 22). Note the heterogenous distribution patterns particularly of the D_1 and D_3 mRNAs.

In regard to the human cerebral cortex, the D_1 family of receptor genes are the most abundantly expressed of all dopamine receptor subtypes (compare Figs. 7–10). The highest levels of the D_1 receptor mRNA is predominantly localized to the infragranular layers V/VI with some cortical regions also showing high levels in the supragranular layer II/III (Meador-Woodruff et al., 1996; Hurd et al., 2001). The laminar pattern of most cortical regions has a similar mRNA expression organization, but there is a tendency for relatively higher expression in the supragranular layer in the frontal and insular cortices, whereas mRNA expression predominates in the infragranular layers of the occipital cortex. In addition to differences in laminar pattern, the D_1 mRNA expression varies between the different neocortical regions. The signal is particularly strong in the medial orbital areas, paraterminal gyrus, insula and parietal cortices. Low expression levels are evident in the inferior and middle frontal cortices. These findings indicate that the D_1 receptor expression differs quantitatively between subcompartments of the frontal neocortex.

There is an intense expression of the D_1 mRNA in the human striatum (Mengod et al., 1991, 1992; Meador-Woodruff et al., 1994b, 1996; Hurd et al., 2001) (Fig. 7). Most cells within the adult human caudate, putamen and nucleus accumbens show high D_1 mRNA expression. The D_1 mRNA is expressed in both the patch/striosome and matrix compartments of the dorsal striatum with a slightly greater expression in the patch neuronal populations. Rat models have shown that the patch/striosome neurons are the first striatal neurons to be born and innervated by dopaminergic terminals (Fishell and Van Der Kooy, 1987; Fishell and van der Kooy, 1991; Song and Harlan, 1994). The

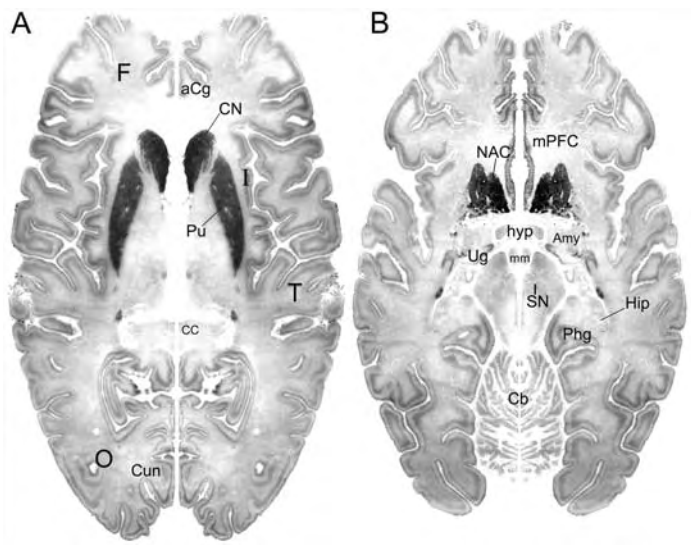

Fig. 7. Anatomical organization of dopamine D_1 mRNA expression in the adult human brain (whole hemisphere horizontal images) at a dorsal (A) and ventral (B) level. Notice strong cortical expression of this dopamine receptor subtype in addition to the intense expression levels in the striatum (CN, Pu and NAc). Adapted from Hurd et al. (2001). aCg, anterior cingulate; Amy, amygdala; Cb, cerebellum; cc, corpus callosum; CN, caudate nucleus; Cun, cuneus; F, frontal lobe; Hip, hippocampus; hyp, hypothalamus; I, insular cortex; mPFC, medial prefrontal cortex; mm, medial mammillary nucleus; NAc, nucleus accumbens; O, occipital lobe; Phg, parahippocampal gyrus; Pu, putamen; SN, substantia nigra; T, temporal lobe; U, uncal gyrus.

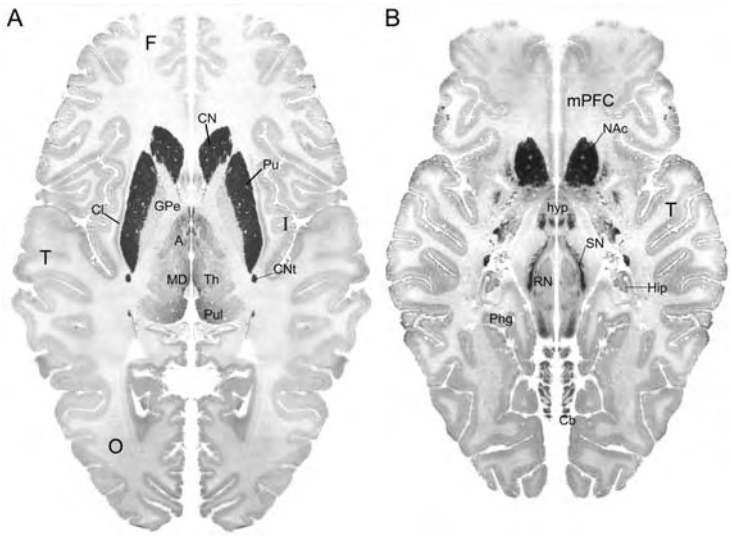

Fig. 8. Anatomical organization of the dopamine D_2 mRNA in the adult human brain (whole hemisphere horizontal images) at a dorsal (A) and ventral (B) level. The D_2 mRNA is predominantly expressed in the striatum, mesencephalon (SN), hypothalamus. Adapted from Hurd et al. (2001). A, anterior thalamus; Cb, cerebellum; Cl, claustrum; CN, caudate nucleus; CNt, tail of caudate nucleus; Cun, cuneus; F, frontal lobe; hipp, hippocampus; hyp, hypothalaus; I, insular cortex; MD, mediodorsal thalamus; mPFC, medial prefrontal cortex; mm, medial mammillary nucleus; NAc, nucleus accumbens; O, occipital lobe; Phg, parahippocampal gyrus; Pu, putamen; Pul, pulvinar; SN, substantia nigra; T, temporal lobe.

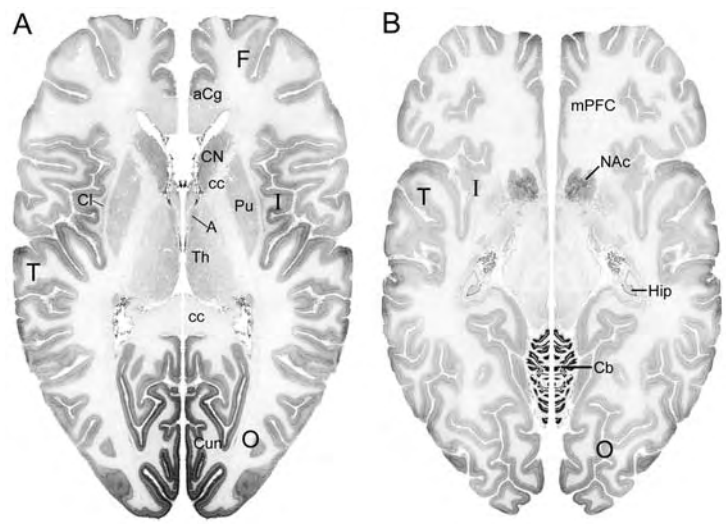

Fig. 9. Anatomical organization of the dopamine D_3 mRNA in the adult human brain (whole hemisphere horizontal images) at a dorsal (A) and ventral (B) level. Note the low expression of the signal in most brain areas except for the occipital cortex, hippocampus (primarily dentate gyrus), and cerebellum (a part of the signal may be unspecific; see Suzuki et al. (1998)). In the striatum the levels are highest in the ventral (NAc) as compared to the dorsal (CN, Pu) striatum. Adapted from Suzuki et al. (1998). A, anterior thalamus; aCg, anterior cingulate; Amy, amygdala; Cb, cerebellum; cc, corpus callosum; Cl, claustrum; CN, caudate nucleus; Cun, cuneus; F, frontal lobe; Hip, hippocampus; I, insular cortex; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; O, occipital lobe; Pu, putamen; T, temporal lobe; Th, thalamus.

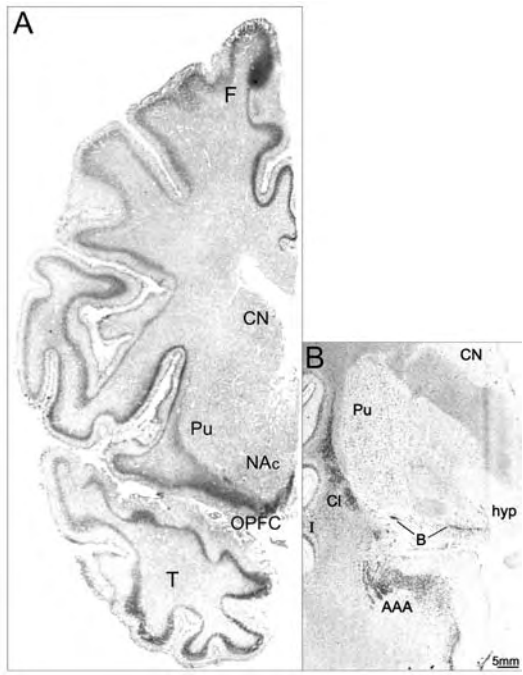

Fig. 10. Anatomical organization of the dopamine D_5 mRNA in the adult human brain (coronal images). The images represent the D_5 expression pattern in a whole coronal hemisphere section at a mid-striatal level (A) and a coronal section at the postcommissural striatal level (B). Note the intense D_5 mRNA expression primarily in the cerebral cortex (e.g. mOPFC and SF). Moderate to strong labeling is also apparent in the claustrum and anterior amygdala nucleus. aCg, anterior cingulate; AAA, anterior amygdala nucleus; B, magnocellular basal forebrain complex; cc, corpus callosum; Cl, claustrum; CN, caudate nucleus; F, frontal; hyp, hypothalamus; I, insular cortex; OPFC, orbital prefrontal cortex; NAc, nucleus accumbens; Pu, putamen; SF, superior frontal; T, temporal cortex.

patch/striosome neurons are also the first striatal cells to project to the substantia nigra (Fishell and van der Kooy, 1991), constituting the 'striosomal' output neurons (primarily expressing the neuropeptides prodynorphin and substance P) as characterized in primates (see Graybiel and Penney, 1999). Within the matrix compartment, the D_1 is primarily within the 'direct' striatal output neurons that express the tachykinin peptide substance P and innervate the substantia nigra pars reticulata and internal segment of the globus pallidus. In contrast to the adult pattern, during early development and prior to birth, the D_1 mRNA synthesis is more intense within cells associated with the patch/striosome compartment thus resulting in a heterogenous striatal distribution pattern of the D_1 signal (Brana et al., 1996; Fig. 6).

In addition to the striatum, the bed nucleus of the stria terminalis and interface islands (apparent homolog to the islands of Calleja in rodents) within the ventral striatum show intense D_1 mRNA expression levels in the human brain (Hurd et al., 2001; Fig. 7). These regions are implicated in limbic function and thus have important relevance for the impaired D_1 transmission in psychiatric disorders. The D_1 mRNA is normally not detected in the thalamus, hypothalamus, hippocampus, pallidum, cerebellum, substantia nigra, pons, raphé and other brainstem nuclei of the normal human brain.

2.3.2. Dopamine D_1 receptor protein

The highest densities of the D_1 dopamine receptors are found throughout the striatum (Fig. 11 shows the dense binding in the caudate nucleus and putamen) (see e.g. Hall et al., 1988; Lidow et al., 1991; Wamsley et al., 1992). There is a heterogenous organization of the D_1 dopamine receptors in the striatum. In the caudate nucleus, the density of the D_1 dopamine receptors is higher in the acetylcholinesterase-poor striosomal compartment as compared to the matrix (Besson et al., 1988; Langer and Graybiel, 1989; Rappaport et al., 1993; Brené et al., 1995). In contrast, D_1 dopamine receptor-rich patch/striosome in the putamen are found mainly in the medial part of this region. The binding density in the striosomes has been estimated to be approximately 25–35% higher than in the surrounding matrix (Besson et al., 1988; Berendse and Richfield, 1993). In one meticulous study (Piggott et al., 1999), the D_1 dopamine receptor density was

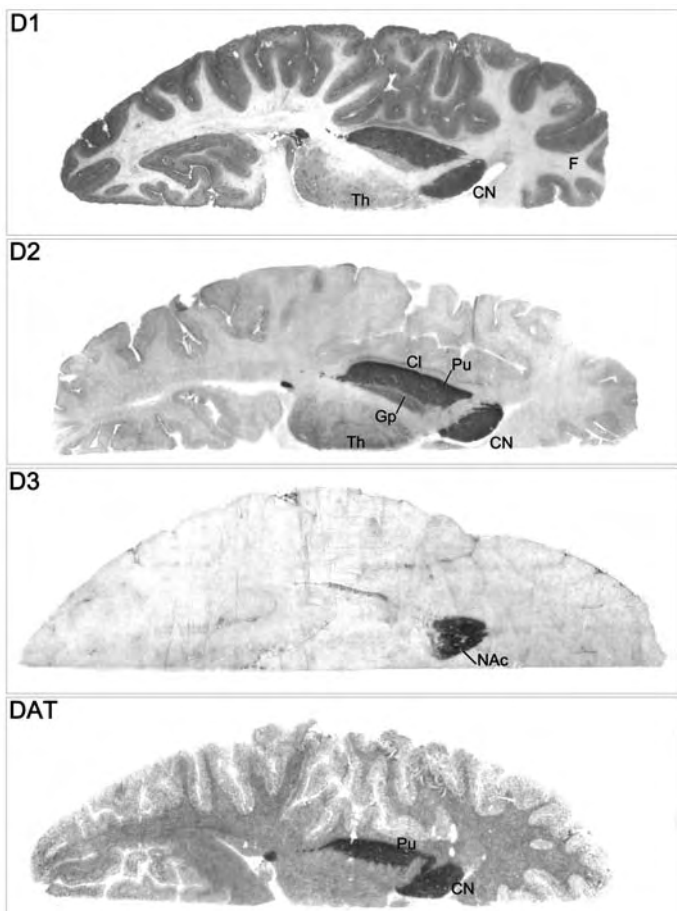

Fig. 11. Whole hemisphere autoradiography showing the distribution of dopamine receptors (D_1–D_3) and the dopamine transporter in the human brain. The D_1 receptor was visualized using [^{3}H]NNC-112, the D_2 receptor using [^{125}I]epidepride, the D_3 receptor using [^{3}H]PD128907 and the transporter using [^{125}I]PE2I. Distribution studies with these radioligands are presented in more detail in references (Hall et al., 1996a, b, 1997) for the D_2, D_3 and dopamine transporter, respectively. CN, caudate nucleus; NAc, nucleus accumbens; Pu, putamen.

shown to have a rostrocaudally declining gradient in the putamen but not in the caudate, such that at levels posterior to the anterior commissure, there was significantly lower D_1 dopamine receptor binding in the putamen compared to the caudate.

In the globus pallidus, D_1 dopamine receptors are mainly within the internal pallidum, and only low densities are present in the external part. Both pars reticulata and compacta of the substantia nigra have a considerable density of D_1 dopamine receptors, although the highest is seen in pars reticulata (Joyce et al., 1986; Besson et al., 1988; Thibaut et al., 1990). The D_1 receptors present in the pallidum and substantia nigra pars are most likely localized on terminal projections since there are no D_1 mRNA-expressing cells in these regions. Ultrastructural characterization of the D_1 receptors has revealed that this dopamine receptor subtype is primarily within the spines and shafts of projection neurons (Bergson et al., 1995). The ultrastructural analyses have also shown that the D_1 receptor is predominantly localized to the dendritic spines of pyramidal cells within the primate cerebral cortex and hippocampus, so dopaminergic signal mediated via D_1 receptors can influence excitatory cells (Bergson et al., 1995).

2.3.3. Dopamine D_2 receptor mRNA expression

D_2 mRNA expression (and binding) are detected from at least week 12 of human ontogeny in the substantia nigra and striatum (Aubert et al., 1997; Brana et al., 1997) as well as in the hippocampal formation and temporal cortex (Gurevich et al., 2000). Many subcortical and cortical cells throughout the brain express abundant levels of the D_2 mRNA as compared to the other dopamine receptor subtypes. The highest expression is found in association with the classic nigrostriatal basal ganglia circuit, namely the substantia nigra pars compacta and striatum (Joyce and Meador-Woodruff, 1997; Hurd et al., 2001) (Fig. 8). Within the mesencephalon, the D_2 mRNA-expressing cells are most abundant in the ventral tier cell groups. The D_2 is considered to function as an autoreceptor modulating dopamine synthesis, cell firing, and release. The lower expression of the dopamine D_2 mRNA in the dorsal tier in contrast to the levels in the ventral tier mesencephalic cell group (Hurd et al., 1994; Meador-Woodruff et al., 1994a; Haber et al., 1995; Gurevich and Joyce, 1999; Fig. 2).

In the adult brain, the D_2 mRNA expression is widespread in the striatum with predominant expression within the matrix, proenkephalin 'indirect' striatal pathway that innervates the external segment of the globus pallidus (see Graybiel and Penney, 1999). D_2 mRNA expression also has a greater association with the matrix compartment during early human development (Brana et al., 1997).

The dopamine D_2 receptor mRNA is expressed in the neocortex of the adult human brain, but with much lower levels as compared to the striatum (Gandelman et al., 1991; Meador-Woodruff et al., 1994, 1997; Hurd et al., 2001). The laminar distribution pattern of the D_2 hybridization signal is more uniform over the cerebral cortex with labeling in both superficial and deep layers. Also among the neocortical regions there is evidence of heterogeneity since relatively high cortical D_2 hybridization signals are detected in the most rostral part of the temporal lobe and also in parts of the parietal and occipital cortices (Fig. 8).

The D_2 mRNA expression in the bed nucleus of the stria terminalis is particularly strong as well as in the magnocellular complex of the basal forebrain including the diagonal band of Broca and the nucleus basalis of Meynert (Gurevich et al., 1997a; Hurd et al., 2001). In contrast to the virtual lack of the D_1 signal in the human

hippocampus, there is significant expression of the D_2 mRNA expression in this region. The highest expression levels are localized to the dentate and uncal gyri of the hippocampus (Meador-Woodruff et al., 1994b; Gurevich et al., 1997a; Hurd et al., 2001), within the granular cell layer (Gurevich et al., 1997a). D_2 mRNA-expressing cells are also evident, though in lower numbers, in the rest of the hippocampal formation with characteristic morphology of pyramidal neurons in the CA region and subiculum (Gurevich et al., 1997a).

The amygdaloid complex also shows a heterogenous pattern of the D_2 mRNA expression. The highest D_2 mRNA expression is found within the basal and lateral amygdala nuclear group (Gurevich and Joyce, 1999; Hurd et al., 2001), a pattern already evident in the midgestation (at least week 17) human fetus (Wang et al., unpublished). D_2 mRNA-positive cells have also been identified in other amygdala nuclei such as the central, lateral and cortical nuclei in the adult brain (Gurevich and Joyce, 1999).

There is a marked differential expression of the D_2 mRNA as compared to other dopamine receptor genes in the human thalamus. In contrast to the apparent lack of D_1 mRNA expressing cells in this structure, most thalamic nuclei express the D_2 mRNA to some extent (Gurevich and Joyce, 1999; Hurd et al., 2001). The highest expression is often detected in the caudal intralaminar nuclei (in particular the parafascicular), paraventricular, intralaminar cell clusters within the internal medullary lamina, and the ventral posterior nuclei. Scattered D_2-expressing cells have also been detected in the mediodorsal nucleus and moderate expression is evident throughout the lateral dorsal, ventral anterior, pulvinar, lateral geniculate and slightly lower in the medial geniculate nuclei. This organization would indicate a rather strong role for D_2 receptor mRNA-expressing neurons in dopamine regulated signaling within the thalamus. There have been differing reports in regard to the presence of the D_2 mRNA in the human subthalamic nuclei – absent (Matsumoto et al., 1996; Augood et al., 2000), weak (Hurd et al., 2001), or moderate (Gurevich and Joyce, 1999) levels of D_2 mRNA expression have all been reported.

The hypothalamus also shows a more preferential expression of the D_2 as compared to the D_1 family of dopamine receptors (Hurd et al., 2001; see Figs. 7–10). D_2 mRNA-expressing cells have been found in most of the major hypothalamic nuclei, in particular the lateral and ventromedial nuclei (Gurevich and Joyce, 1999). Strong expression of the D_2 mRNA has also been detected in the premammillary nucleus (Hurd et al., 2001).

2.3.4. Dopamine D_2 receptor protein

Similar to the pattern of the D_2 mRNA expression, the highest D_2 dopamine receptor densities are found in the caudate nucleus and putamen as demonstrated by in vitro homogenate (Seeman, 1980; Hall et al., 1988, 1994) and in vitro autoradiography (Kessler et al., 1993; Hall et al., 1994; Piggott et al., 1999) studies. There is no clear-cut subregional or patch/matrix-like receptor distribution of D_2 dopamine receptors as evident for the D_1 dopamine receptors, although some authors claim a higher density in the matrix than in the striosomes (Joyce and Murray, 1993; Piggott et al., 1999). However, a lateral to medial gradient in receptor density may be seen, and the density increases slightly rostrocaudally (Piggott et al., 1999). The receptor density is similarly high in nucleus accumbens (Berendse and Richfield, 1993; Kessler et al., 1993), so there is no clear delineation between the nucleus accumbens and caudate nucleus or putamen. The density of D_2

dopamine receptor in the lateral pallidum is weaker (approximately 25% of putamen) than in the caudate nucleus or putamen (Hall et al., 1996a). Very low densities of D_2 dopamine receptors are found in the medial pallidum (Hall et al., 1996a).

With the exception of substantia nigra, much lower densities of the D_2 dopamine receptors are seen in extra-striatal regions, and high affinity radioligands are required to visualize the receptors (Bischoff et al., 1980; Lidow et al., 1989; Hall et al., 1991; Kessler et al., 1993). D_2 dopamine receptors are almost absent from the cerebral cortical regions (generally lower than 0.3% of that in the caudate and putamen (Lidow et al., 1989; Hall et al., 1996a)). The highest cortical density is found in the temporal cortex, with a relatively dense labeling of [^{125}I]epidepride observed throughout the lobe (Hall et al., 1996a). The highest labeling in the temporal cortex is in the superficial layers, although the deeper layers are also more densely labeled than other cortical regions. Goldsmith and Joyce (1996) described the laminar distribution of the D_2 dopamine receptors in the temporal cortex using [^{125}I]epidepride binding and also found the highest D_2 receptor densities in layers I–II. However, in the rostro-caudal part of the temporal cortex, they demonstrated columns of D_2 dopamine receptor enriched bands with substantially higher densities in laminae III and V than in the adjacent parts (Goldsmith and Joyce, 1996). In most regions of the occipital cortex, the distribution of the D_2 dopamine receptor is mainly in the superficial layer (Hall et al., 1996a). However, in a distinct part of the rostro-medial occipital cortex, increasing in length toward the basal occipital cortex, the receptors are found mainly in a deep layer, which probably corresponded to layer V, whereas virtually no receptors are found in the superficial layers (Hall et al., 1996a). In the medial prefrontal cortex, short bands of layer V are labeled with [^{125}I]epidepride, although somewhat weaker than in the occipital cortex. The labeling in the striate cortex is complex, with dense labeling in distinct subregions of both the superficial and deep laminae (Hall et al., 1996a). There is low or very weak D_2 dopamine receptor density in archicortical regions, such as the hippocampus including the dentate gyrus (Bischoff et al., 1980; Hall et al., 1996a). In the amygdaloid complex, low but significant densities of D_2 dopamine receptor are observed in, e.g. the amygdalostriatal transition and basolateral amygdala (Hall et al., 1996a).

The distribution of D_2 dopamine receptors in the thalamus is very heterogeneous. In some thalamic nuclei, such as the dorsomedial, centromedial and anteroprincipal nuclei, the D_2 dopamine receptor density is relatively high, whereas the density is much lower in other thalamic regions (e.g. medial geniculate, centromedial and pulvinar) (Hall et al., 1996a). Low density is also apparent in the mediodorsal and this region is circumscribed by a thin layer of dense labeling consistent with the internal medullary lamina. The different nuclei of hypothalamus show only weak labeling with [^{125}I]epidepride. A slightly higher density of [^{125}I]epidepride binding sites is seen in the septum (Hall et al., 1996a).

Ultrastructural characterization of the D_2 receptors have revealed that they are often present within the GABA-containing cells within the striatum, cerebral cortex, hippocampus, globus pallidus and thalamic reticular nucleus (Mrzljak et al., 1996). Thus, dopaminergic transmission via the D_2 receptors predominantly provides inhibitory local intrinsic control in the cerebral cortex and inhibitory regulation of the projection pathways.

2.3.5. Dopamine D_3 mRNA expression

A limited number of human brain structures express very intense D_3 mRNA, namely the islands of Calleja (Suzuki et al., 1998), ventral striatum (Landwehrmeyer et al., 1993;

Suzuki et al., 1998; Gurevich and Joyce, 1999) and the granular cell layer of the dentate gyrus (Landwehrmeyer et al., 1993; Suzuki et al., 1998) (Fig. 9). During development, at least the midgestational human fetal stage, the D_3 mRNA expression shows a patchy heterogenous organization even extending up to the dorsal striatum (Fig. 6). Moderate expression of the D_3 is also present within the mesencephalon dopamine cell groups, preferentially in the ventral tier, although the dorsal tier appears to have some sporadic rare D_3 mRNA-expressing cells (Meador-Woodruff et al., 1994a; Suzuki et al., 1998; Gurevich and Joyce, 1999). Neurons expressing the D_3 mRNA are also evident in the thalamus, in particular the anterior and lateral geniculate nuclei with lower levels in the medial geniculate, pulvinar and mediodorsal nuclei (Suzuki et al., 1998; Gurevich and Joyce, 1999). Hypothalamic expression of the D_3 mRNA is predominantly within the mammillary body (Landwehrmeyer et al., 1993; Suzuki et al., 1998; Gurevich and Joyce, 1999), with positive-expressing cells also detected in the medial preoptic, dorsal, lateral, posterior nuclei (Gurevich and Joyce, 1999). In general, low levels of the D_3 transcript is present throughout the entire cortical mantle (Meador-Woodruff et al., 1994b; Suzuki et al., 1998) with slightly higher abundance in the subcallosal (medialorbital prefrontal), anterior cingulate and visual cortices (Suzuki et al., 1998). In addition to the dentate gyrus, the D_3 mRNA is expressed in much lower levels in the pyramidal cells of the CA region and subiculum within the hippocampal formation (Meador-Woodruff et al., 1994b; Suzuki et al., 1998). The D_3 mRNA expression in the amygdaloid complex is low with the 'most abundance' signal in the cortical and basolateral nuclei (Suzuki et al., 1998; Gurevich and Joyce, 1999). Overall, the association of the D_3-expressing cells within the ventral striatum, as well as in the hippocampo-mammillo-thalamo-cingulo-hippocampal circuit, is consistent with a contribution of the D_3 receptor to limbic-related functions.

2.3.6. Dopamine D_3 receptor protein

The first D_3 dopamine receptor-selective compound was the agonist 7-OH-DPAT (Lévesque et al., 1992) and this compound has been used in a number of binding, autoradiographical and behavioral studies (Daly and Waddington, 1993; Damsma et al., 1993; Ahlenius and Salmi, 1994; Herroelen et al., 1994). The more recent data have indicated that 7-OH-DPAT also interacts with the D_2 dopamine receptor (Gonzalez and Sibley, 1995). With the development of the radioligand [^{3}H]PD128907, a new selective D_3 dopamine receptor agonist with an 18–40-fold selectivity for D_3 over D_2 dopamine receptors, it has become possible to further characterize the D_3 dopamine receptor (Dijkstra et al., 1988; De Wald et al., 1990; Akunne et al., 1995; Pugsley et al., 1995). The D_3 dopamine receptors are localized primarily in limbic regions, such as the nucleus accumbens (Fig. 12) and Islands of Calleja (Herroelen et al., 1994) consistent with its mRNA expression pattern (see Section 2.3.5). There is a lower, but quite significant, density also evident in the ventral parts of the caudate nucleus and putamen (Hall et al., 1996b; Fig. 12). The D_3 dopamine receptor distribution in the striatum is reported to be heterogeneous, with higher density of binding sites in patch/striosome compartment than in the matrix (Murray et al., 1994; Hall et al., 1996b; Piggott et al., 1999). In the rodent brain, there is a higher D_3 dopamine receptor density in the 'shell' than in the 'core' subregion of the nucleus accumbens (Diaz et al., 1994; Booze and Wallace, 1995), a difference that is not as distinct in the human accumbens (Voorn et al., 1994; Hall et al., 1996b), even though there does tend to be a slight tendency for higher D_3 binding in the shell-like region (Fig. 12).

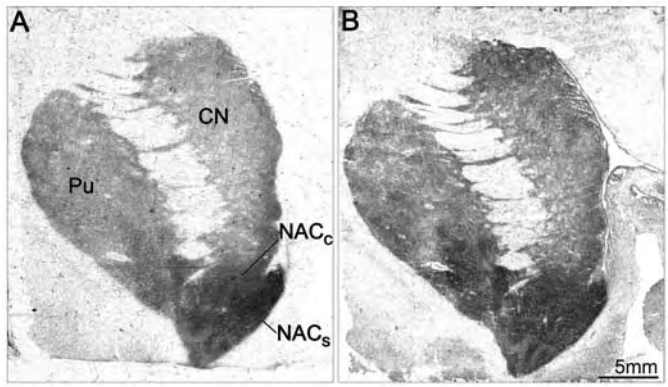

Fig. 12. Dopamine D_3 binding sites in the human striatum as revealed using [^{3}H]7-OH DPAT (A) and [^{3}H]PD128907 (B). Note the heterogenous binding pattern in the ventral striatum (nucleus accumbens) including the ventral caudate and putamen; the heterogeneous signal in the dorsal striatum was most apparent using the PD128907 ligand. Slightly higher binding (more visible with the 7-OH DPAT ligand) was also apparent in the more medial 'shell-like' areas as compared to the adjacent 'core-like' region in the ventral striatum. CN, caudate nucleus; NAc_c, nuclues accumbens core; NAc_s, nucleus accumbens shell; Pu, putamen.

Distinct, but weak D_3 dopamine receptor binding is seen in the substantia nigra (Hall et al., 1996b). Very low densities of the D_3 dopamine receptor are apparent in cerebral cortex (Hall et al., 1996b). The D_3 dopamine receptor is also present in low amounts in lobule 10 in the human cerebellum (Herroelen et al., 1994; Wallace and Booze, 1995). In a comparative study using [^{3}H]PD128907, Levant et al. (Levant and Desouza, 1993) demonstrated the D_3 dopamine receptors in the rat cerebellum, but failed to show binding in the human cerebellum.

2.3.7. Dopamine D_4 mRNA expression

Very limited information is known about the anatomical organization of the D_4 receptor cells populations in the human brain. It has been documented that the highest expression of the D_4 gene is within the cerebral cortex throughout layers II–IV with a greater abundance in the deep cortex laminae (Meador-Woodruff et al., 1994b, 1996). Expression of D_4 mRNA has been detected in the hippocampal formation with the greatest abundance of expression in the granular layer of the dentate gyrus and more modest expression in the CA regions (Meador-Woodruff et al., 1994b). In contrast to the D_2 and D_3 receptors, striatal expression of the D_4 mRNA is questionable (Meador-Woodruff et al., 1996).

2.3.8. Dopamine D_4 receptor protein

Till date, there exist, no reliable and widely used radioligands or systems for the study of the D_4 dopamine receptors. However, a number of attempts have been made using various pharmacological blockers to selectively label the D_4 dopamine receptors. Seeman et al. (1993) used an indirect method, by comparing the densities of the two radioligands [^{3}H]nemonapride (with a high affinity for D_2 dopamine and D_4 dopamine receptors) and [^{3}H]raclopride (with a high affinity for D_2 dopamine, but low for the D_4 dopamine receptors). A similar approach has been used by others (Murray et al., 1995).

However, these results have been questioned, and are not considered reliable (Seeman and Van Tol, 1995). More specific radioligands have been developed recently. Using the new D_4 dopamine receptor radioligand [^{3}H]NGD-94-1, D_4 dopamine receptors were identified in the hippocampus, hypothalamus, dorsal medial thalamus, entorhinal cortex, insular cortex, prefrontal cortex and lateral septal nucleus (Primus et al., 1997; Lahti et al., 1998). In contrast to the distribution of D_1–D_3 dopamine receptors, no binding was seen in the basal ganglia. These results correspond to the distribution of D_4 dopamine receptor mRNA.

Immunohistochemical analyses have provided evidence of D_4 receptors in medium-sized spiny neurons in the human striatum (Khan et al., 1998). Using immunohistochemistry, D_4 dopamine receptors were also localized within the GABA-containing cells of the striatum as well as GABAergic cells in the cerebral cortex, hippocampus, globus pallidus, substantia nigra and thalamic reticular nucleus of the nonhuman primate (Mrzljak et al., 1996). Thus, dopaminergic transmission via the D_4 dopamine receptor appears to be predominantly inhibitory, so that blockade of D_4 dopamine receptors may result in disinhibition of excitatory transmission in intrinsic cortical, thalamocortical and extrapyramidal pathways.

2.3.9. Dopamine D_5 mRNA expression

In contrast to the other dopamine receptor genes, the D_5 receptor mRNA shows a predominant cortical expression in the human brain (Meador-Woodruff et al., 1994b, 1996; see Fig. 9). High expression of the D_5 mRNA is evident in a band predominantly within deep laminae; layer II also expresses the D_5 mRNA (Meador-Woodruff et al., 1996). Lower expression of the D_5 has been detected in the dentate gyrus granular layer as well as in the CA regions (Meador-Woodruff et al., 1994b). The striatum also shows low D_5 mRNA expression with positively-labeled cells scattered throughout the region. Other forebrain structures showing expression of the D_5 mRNA include the claustrum, magnocellular basal forebrain complex, anterior amygdala nucleus, amygdalostriatal transition area and periamygdala cortex, as well as the parafascicular nucleus and internal medullary lamina of the thalamus. Despite scattered expression of D_5 mRNA in subcortical structures, overall, the most profound expression of the D_5 gene in humans is seen throughout the entire cortical mantle, in particular the medial and orbital prefrontal, superior frontal and temporal cortices (Fig. 10).

2.3.10. Dopamine D_5 receptor protein

No selective radioligand for the study of the D_5 dopamine receptor has been developed so far. All compounds binding to the D_1 dopamine receptor have an affinity for the D_5 dopamine receptor also, and there exists no pharmacological discriminant tool that can selectively label any of these receptor subtypes using receptor binding techniques.

Ultrastructural studies of the D_5 dopamine receptor using immunocytochemistry have revealed that this receptor subtype is highly expressed in the human cortex in pyramidal neurons and their dendrites are present within layers IV–VI (Khan et al., 2000). The D_5 dopamine receptor is also localized to the striatum, substantia nigra (both pars compacta and reticulata), the superior colliculus, the thalamus and the pyramidal cells of hippocampus (Khan et al., 2000). In the striatum, electron microscopic analysis indicates that D_5 dopamine receptors are present in the spines where asymmetric synapses are formed

with afferent axons (Khan et al., 2000). Such an arrangement suggests the involvement of an D_5 dopamine receptor in modulating incoming excitatory input to the striatum. The D_5 dopamine receptor is also localized in the dendrites and spines of striatal neurons primarily within extended long dendrites of medium spiny projection neurons and large cholinergic interneurons (Khan et al., 2000).

Overall, with regard to the organization of dopamine receptors in the human brain, it appears that, with an exception of the D_4 receptor, neurons expressing D_1-like (and particularly the D_5) receptors have a predominant cortical localization, whereas the D_2 and D_3 receptors-expressing cells have a stronger subcortical presence.

2.4. DOPAMINE TRANSPORTERS

The neuronal dopamine transporter (DAT) is a presynaptically located protein responsible for reuptake and thus removal of dopamine from the synaptic cleft. Dopamine transport carriers provide one of the most important means by which the actions of synaptic (and extrasynaptic) dopamine are terminated in the brain. As the DAT is exclusively located on terminals of dopamine neurons (reviewed in Boja et al., 1994), this transporter has served as a good anatomical dopaminergic marker.

2.4.1. DAT mRNA expression

Expression of the DAT gene has been documented in the human mesencephalon from at least week 12 of human fetal life (Aubert et al., 1997). By week 19, the DAT mRNA is predominantly expressed in the substantia nigra pars compacta with lower signals in the ventral tegmental area similar to the organization in the human adult. As evident in Fig. 2, the expression of DAT mRNA has a similar anatomical distribution in the mesencephalon to that of D_2 mRNA. The highest expression of DAT mRNA is within the ventral tier mesencephalic cell group. The ventral tegmental and retrorubral areas express moderate levels of the DAT mRNA and the dorsal cell group of the substantia nigra pars compacta expresses very low levels (Fig. 2). This pattern is consistent with the organization observed in the monkey brain (Haber et al., 1995). The strong overlap between DAT mRNA and protein in dopaminergic neurons and their processes was earlier thought to indicate a predominantly synaptic mode of dopamine neurotransmission. However, some ultrastructural analyses have now revealed that the DAT is also localized outside the synaptic area.

2.4.2. DAT protein

In the human brain, DAT immunoreactive cell bodies and dendrites are enriched primarily in the ventral tier and lateral dorsal tier mesencephalic cell groups and dense DAT immunoreactive fibers are present in the dorsal and ventral striatum (Ciliax et al., 1999). However, DAT immunoreactivity and binding is lower in the patch/striosome versus the matrix striatal compartment and relatively lower in the ventral as compared to the dorsal striatum. There is also a heterogeneity of the distribution within the human nucleus accumbens with lower densities of the DAT protein (and binding) in the shell as compared to the core subregions. The lower levels of the DAT within dopaminergic axons would suggest a greater dopamine diffusion in these regions. Only one study to date has examined the ultrastructural localization of the DAT in the primate

brain. It was observed that DAT-labeled processes in the dorsolateral caudate nucleus frequently had large, synaptic vesicles or formed synapses (Lewis et al., 2001). In contrast, in the rodent, the DAT is not concentrated near synapses in either the dorsal (Hersch et al., 1995, 1997) or ventral (Nirenberg et al., 1997) striatum suggesting a predominant extrasynaptic means of dopamine reuptake. The ultrastructural organization of the DAT may differ between species, but further studies are necessary.

DAT immunoreactive fibers are also evident in mesocorticolimbic terminal sites, such as the amygdala (primarily basolateral and central with sparse fibers in the lateral and basomedial) (Ciliax et al., 1999; Freedman and Shi, 2001) and cerebral cortex (e.g. prefrontal, entorhinal, insular) (Ciliax et al., 1999; Lewis et al., 2001). The DAT is, however, also expressed in the visual cortex concordant with the documented more widespread cortical dopaminergic innervation that is evident in primates (see Section 2.2). Dense DAT-immunoreactive fibers are apparent in the lateral, ventral region of the bed nucleus of stria terminalis (Freedman and Shi, 2001). DAT immunoreactive axons are also present in the mediodorsal and anterior thalamus (Melchitzky and Lewis, 2001). Consistent with the observations from in situ hybridization studies, only a small subpopulation of mesencephalic dopamine neurons (namely, those in the medial ventral tegmental area) express DAT immunoreactivity, whereas hypothalamic dopamine neurons express very little, if any, DAT immunoreactivity (Ciliax et al., 1999). Only few DAT-immunoreactive axons have been identified in the hippocampal CA fields and subiculum (Lewis et al., 2001), but there is a high density of DAT-immunoreactivity present in the outer two-thirds of the molecular layer of the dentate gyrus, which receive entorhinal cortex input via the perforant pathway. In the primate hippocampus, the DAT is most frequently associated with synaptic structures, whereas, in contrast, the DAT in the cerebral cortex is usually located at a distance from the synaptic sites of DA release and more closely apposed to dendrites (Lewis et al., 2001). This organization would suggest that cortical dopamine release has a greater possibility to act at DA receptors not in the immediate vicinity of the DA terminals, leading to a greater extrasynaptic action of DA in the human cerebral cortex.

In regard to receptor binding, the highest density of DAT binding sites is found in the basal ganglia (putamen, nucleus caudatus, nucleus accumbens) of the human brain (Boja et al., 1994; Staley et al., 1994a; Hall et al., 1999). The density of dopamine uptake sites has an increasing rostrocaudal gradient in the caudate, especially ventrally, but not in the putamen, where binding is more constant (Staley et al., 1995; Piggott et al., 1999). However, others have failed to show this gradient (Hall et al., 1999; Villares and Stavale, 2001). The compartmentalization of the DAT within the basal ganglia nuclei is not clear. In studies of [^{3}H]mazindol binding in cats and monkeys, DAT density was higher in the matrix than in the striosomes (Graybiel and Moratalla, 1989). Using [^{125}I]PE2I, the distribution of DAT binding sites in the human brain was found to be homogenous in the putamen and caudate nucleus and no differential distribution into the striosomes was seen (Hall et al., 1999). Differences in the pattern of the DAT binding sites that have been observed in various studies might relate to the ligand used to visualize the DAT binding sites, such that aspects such as affinity state may play a role in the apparent discrepant results.

With regard to extrastriatal areas, there is distinct, but much weaker, binding in the substantia nigra, whereas other subcortical structures such as the thalamus have either no, or a very low density of, DAT. Low densities of the DAT are present in the external segment of the globus pallidus and the lateral nucleus of the amygdala (Hall et al.,

1999). There is no apparent DAT binding sites in neocortical regions, hippocampus, or cerebellum.

3. THE ROLE OF THE DOPAMINE SYSTEM IN ADDICTION AND PSYCHIATRIC DISORDERS

3.1. DOPAMINE SYSTEMS IN PSYCHOSTIMULANT ADDICTION

The dopamine system has been closely linked with addictive disorders, especially related to stimulant drugs. These conclusions have in large part been based on animal studies which showed that disruption of the mesocorticolimbic dopamine system abolish stimulant self-administration (Roberts et al., 1977, 1980; Pettit et al., 1984) and that most drugs of abuse acutely elevate dopamine levels in the nucleus accumbens (Imperato and Di, 1986; Imperato et al., 1986; Pontieri et al., 1995). The primary target of psychomotor stimulant drugs, such as cocaine, amphetamine and methylphenidate are the biogenic amine transporters (see Kuhar et al., 1990; Hitri et al., 1994a). Of the biogenic amines, the dopamine neuronal populations have been predominantly investigated in experimental animal and human studies in association with the effects of stimulant agents. Stimulant drugs elevate dopamine levels by (1) blocking the DAT, thereby preventing the reuptake of dopamine released (via exocytosis) from storage vesicles (e.g. cocaine and methylphenidate), and/or (2) releasing dopamine via reversal of the DAT (e.g. amphetamines) (see Amara and Kuhar, 1993; Hitri et al., 1994a). Most studies have focused on the effects of stimulant drugs in the striatum (in particular the ventral striatum) based on the abundance of dopaminergic terminals in this structure, and evidence that this region is important for the integration of information related to reward, motivation, cognition and motor function. A growing body of evidence from human investigations has now provided specific information about impairments of the dopamine systems in relation to the use of psychostimulant drugs also in primates, including man.

3.1.1. In vivo characterization

Nonhuman primate studies have shown that, similar to the extensive data accumulated from the rodent experimental models, administration of psychostimulant drugs, such as cocaine or amphetamine results in the elevation of striatal dopamine levels (Laruelle et al., 1995, 1997; Drevets et al., 1999; Bradberry et al., 2000; Czoty et al., 2002). Extrastriatal alterations in the dopamine levels have also been detected in vivo in monkeys. Moreover, it is now feasible to monitor in vivo dopaminergic responses in the human brain, and the subsequent neural adaptations on the various dopamine-related markers, including DATs and receptors (see e.g. Drevets et al., 2001; Martinez et al., 2003). Human PET studies have been able to determine changes in endogenous dopamine concentrations by estimating the displacement of the low affinity D_2 receptor radioligand, [^{11}C]raclopride, which competes with endogenous dopamine for binding to the D_2 receptor (Hume et al., 1992; Dewey et al., 1993; Volkow et al., 1994; Breier et al., 1997), and to visualize presynaptic dopamine terminals using selective DAT radioligands (Wong et al., 1993; Kuikka et al., 1998; Laakso et al., 1998; Poyot et al., 2001).

A large body of in vivo imaging data has now been accumulated regarding dopamine receptors and DATs in human stimulant users. The majority of these studies have been

carried out by Volkow and colleagues showing specific anatomical and temporal alterations within the dopaminergic system in stimulant users (Volkow et al., 2001b). Such human studies have revealed that there is a strong involvement of the D_2 receptor in the perception of the reinforcing effects of psychostimulant drugs. Methylphenidate-induced 'high' was shown to be associated with increases in striatal dopamine levels in normal human subjects (Volkow et al., 1999b). Individuals who perceived the most intense 'high' generally had the strongest release in dopamine levels. In fact, the striatal levels of the dopamine D_2 receptor appears to predict the response to psychostimulants such that normal subjects with low D_2 receptors experience methylphenidate as pleasant, whereas those with high D_2 receptors experience the stimulant to be unpleasant (Volkow et al., 1999c; Fig. 13). Acute elevation of in vivo striatal dopamine levels following administration of amphetamine in humans has also been shown to be correlated to the euphoric properties of the stimulant drug (Drevets et al., 2001; Martinez et al., 2003). A clear regional specificity of the dopaminergic response to stimulant use has also been observed in humans with the greatest alterations apparent within the ventral striatum (associated with limbic function) and the postcommissural putamen (associated with sensorimotor function) (Martinez et al., 2003).

In vivo the PET studies have revealed that low D_2 receptor availability is a common feature of many drugs of abuse, e.g. cocaine (Volkow et al., 1990, 1997),

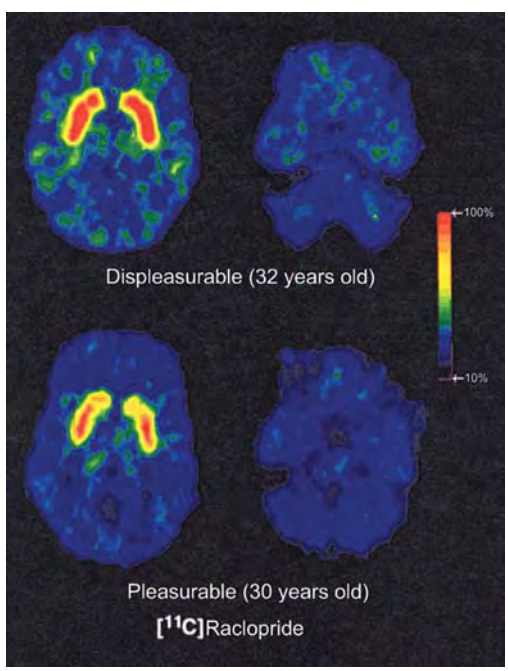

Fig. 13. In vivo PET images from Volkow and colleagues (1999b) showing a correlation between the rewarding effects of stimulant drug (intravenous 0.5 mg/kg methylphenidate) and the level of striatal dopamine. The images show the distribution volume of [^{11}C]raclopride binding at the levels of the striatum (left) and cerebellum (right) in a healthy male subject who reported the effects of methylphenidate as pleasant and in a healthy male subject who reported them as unpleasant. Note the lower activity (reflecting increased dopamine levels) in the striatum of the subject who reported the effects of methylphenidate as pleasant than in the striatum of the subject who reported them as unpleasant.

methamphetamine (Volkow et al., 2001b), heroin (Wang et al., 1997) and alcohol (Hietala et al., 1994; Volkow et al., 1996a). The low D_2 receptor availability in substance abusers might also be a consequence of the neural adaptations to the repeated drug use. Such low D_2 receptors may contribute in part to the repeated use of drugs by favoring pleasant responses in abusers with this low D_2 receptor binding. There is evidence that low in vivo D_2 receptor availability is correlated with the frequency of the A1 allele of the human D_2 gene in normal subjects (Pohjalainen et al., 1998). Mutations in the promoter/regulatory gene element could affect the dopamine D_2 expression and thus the vulnerability to abuse drugs. The involvement of the D_2 receptor in drug reinforcement is also substantiated by animal studies in which the pre- and postdrug history is known and baseline predrug levels of the in vivo dopamine markers can be established. For example, non-human primates with low D_2 binding, as measured in vivo with PET, have been shown to self-administer cocaine more readily than animals with normal in vivo D_2 binding (Morgan et al., 2002). Moreover, the monkeys who self-administered cocaine to a much greater extent were subordinate in the social group housing, whereas those with high D_2 binding were the dominant monkeys which did not readily self-administer cocaine (Morgan et al., 2002). Another interesting observation from this investigation was that prior to the social stratification and access to cocaine, the PET evaluation had revealed that both the eventual subordinate and dominant monkeys had similar in vivo D_2 binding levels. Thus, it appears that environmental factors (e.g. social stress) can lead to changes in D_2 occupancy that can subsequently alter the vulnerability to self-administer drugs of abuse.

Although the synaptic dopamine levels are elevated following the acute drug use in non-dependent subjects, there is also an indication that there is decreased dopamine responsiveness in human cocaine abusers (Volkow et al., 1997). Such reductions in dopamine might contribute to the continued drug-usage in an effort to reestablish normal dopamine levels.

In addition to in vivo D_2 receptor occupancy, the degree of DAT blockade had also been implicated in predicting the intensity of the 'high' induced by stimulant drugs. However, dopamine blockade by itself is not sufficient to account for the subjective 'high'. DAT blockade $>50\%$ has been shown to be necessary, but not sufficient, to induce a 'high' in response to stimulant drugs (Volkow et al., 1999d). For example, oral methylphenidate, which leads to slow DAT blockade, does not induce a 'high', even at doses which block more than 60% of the DAT (Volkow et al., 1996b, 1999a). Moreover, there are individuals who fail to experience a cocaine-induced 'high' despite significant blockade of the DAT (Volkow et al., 1999d). The rate at which the DATs are blocked might contribute more to the experience of the 'high' (Volkow et al., 2000).

As mentioned above, reduced dopamine D_2 receptor availability is a characteristic feature of stimulant abusers. This reduction in the dopamine D_2 receptors is not only evident in the striatum, but also in limbic lobe structures, e.g. cingulate gyrus and orbitofrontal cortex, and are associated with decreased metabolism (Volkow et al., 1992, 1993). Dysfunction of the frontal-striatal circuitry, primarily the caudate, putamen and ventral prefrontal (including the anterior cingulate) cortex is a key feature of obsessive compulsive disorder (see Rauch et al., 2001; Rosenberg et al., 2001) and disruption of these dopaminergic systems in human cocaine abusers might contribute to the compulsive drug intake behavior characteristic of drug addiction. In fact, recent magnetic imaging has revealed that there is significant gray matter reduction in the limbic-related cortical (orbitofrontal, anterior cingulate, insular and superior temporal) areas of cocaine-dependent subjects (Franklin et al., 2002). Taken together, these findings suggest

neurochemical, functional and structural deficits in discrete brain regions associated with the mesocorticolimbic dopaminergic system.

An important feature of in vivo imaging studies is the ability to conduct longitudinal repeated studies in the same individual. The PET studies have now revealed long-term neural adaptations of D_2 and DAT dopaminergic markers as a consequence of psychostimulant abuse over the course of the addictive behavior. Persistent reduction of the D_2 receptors have been revealed in detoxified cocaine-dependent subjects which were correlated with self-reports of dysphoria (Volkow et al., 1990, 1993). Consistently, reduced DAT density has been documented in vivo in nonviolent (type I) alcoholics (Tiihonen et al., 1995). PET studies of methamphetamine abusers have also reported significant impairment (decrease) of DAT binding in the caudate and putamen that persists after long abstinence periods (McCann et al., 1998; Sekine et al., 2001; Volkow et al., 2001a, 2001c) implicating potential damage of the dopamine nerve terminals. Reduced DAT levels in detoxified methamphetamine abusers are associated with motor and cognitive impairments; the lower the in vivo DAT levels, the worse the subject's performance (Sekine et al., 2001; Volkow et al., 2001c). For example, reduced DAT density, as imaged by PET, in the ventral striatum and caudate-putamen was found to be directly associated with the duration of methamphetamine use and the severity of persistent psychiatric symptoms (Sekine et al., 2001). Although the effects of stimulant drugs on the dopaminergic system are long lasting, there is evidence in human abusers that there is recovery of the presynaptic dopamine markers with time (Volkow et al., 2001a; Fig. 14). However, the recovery of striatal DAT levels with protracted abstinence in methamphetamine abusers is not correlated with a complete recovery of motor function and cognitive skills (Volkow et al., 2001a). The recovery of DATs following abstinence was directly correlated with the

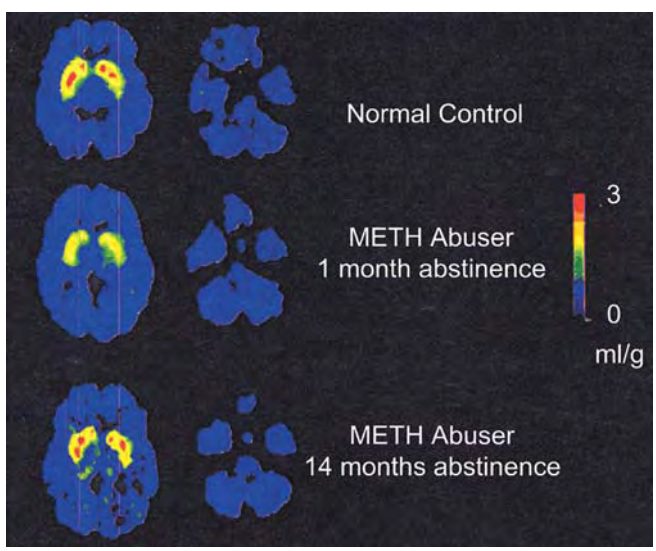

Fig. 14. In vivo PET images from Volkow et al. (2001a) of the distribution volume of [^{11}C]*d-threo*-methylphenidate (label dopamine transporter sites) in a control and a methamphetamine abuser. The images represent the level of the striatum (left) and the cerebellum (right) in a normal control and a methamphetamine abuser evaluated twice, during short and protracted abstinence. Note the reduction of striatal dopamine transporter binding following early abstinence and the reversal to more 'normal' levels in binding in the methamphetamine abuser with protracted abstinence.

dose and years of methamphetamine use (Volkow et al., 2001a). Thus, it is becoming more evident that although repeated use of stimulant drugs has marked detrimental effects on dopaminergic neuronal populations, there is perhaps a possibility to renormalize some neural systems after prolonged abstinence from the drugs. Further studies are needed to determine when, if ever, during protracted abstinence there is a correlative recovery of function with the in vivo neurochemical markers.

Most PET studies targeted at the dopamine systems have examined the DAT and D_2 receptors, and little information is currently available regarding other DA receptors. Despite the clear involvement of the D_2 receptors in the short and long-term effects of stimulant drugs, one cannot discount the possible contribution of, e.g. D_1 receptor dysfunction in human cocaine in the absence of comparable PET studies. The lack of information about other dopamine receptors is due in part to the lack of suitable radioligands that can be used for in vivo PET. Recent development of D_1-specific radioligands for human studies should hopefully expand our understanding of the D_1 system in drug addiction.

3.1.2. Postmortem characterization

Repeated use of stimulant drugs in humans is associated with reductions of the striatal levels of dopamine (Wilson et al., 1996a) and tyrosine hydroxylase (Wilson et al., 1996a) as well as reductions of dopamine levels in the frontal cortex (Little et al., 1996). Alteration in gene expression is an important mechanism through which long-term effects are maintained in the brain and the compulsive, repeated use of addictive drugs implies impairments at the levels of gene expression. The postmortem studies of human cocaine and amphetamine users have revealed reduction of the mRNA expression levels of the DA transport carriers (Wilson et al., 1996b; Little et al., 1998b). Decreased DAT mRNA expression is also found in association with cocaine-related deaths in which the subjects had documented preterminal 'cocaine psychosis' evident by excited delirium (Chen et al., 1999). Recently, reduction of the transcription factor Nurr1 mRNA and protein levels, was detected in human cocaine (Bannon et al., 2002). Nurr1 modulates the transcription of the DAT gene (Sacchetti et al., 1999, 2001). Based on these findings it would appear that reduced transcription of the DAT leads to a reduction of the DAT protein and binding. Whereas significant reductions of the DAT protein (as measured by Western blot) have been found in human stimulant users (Wilson et al., 1996a, 1996b), there have been inconsistent findings regarding the DAT binding sites in relation to the adaptation to stimulant use in humans. Striatal reductions (Hurd and Herkenham, 1993; Fig. 15), elevations (Little et al., 1993, 1998b; Staley et al., 1994b), or no significant change (Wilson et al., 1996a, b; Little et al., 1998a) of the DAT binding have been documented in human stimulant users. A reduced density of DAT sites has also been reported in the frontal cortex of human cocaine users (Hitri et al., 1994b). These discrepancies may relate to the ligands used in each study to label the DAT that might reveal different affinity and conformational states of the DAT.

Overall, the greatest changes documented for DAT binding in the striatum have been localized to the dorsal subregion. Primate studies where the time course of neurochemical markers can be studied have shown that increased dose and longer duration of cocaine exposure is associated with a progression of the DAT alterations (increase) from the ventral to the dorsal striatal regions (Letchworth et al., 2001). This topographic progression in the alteration of the ventral to dorsal striatal DAT binding is consistent

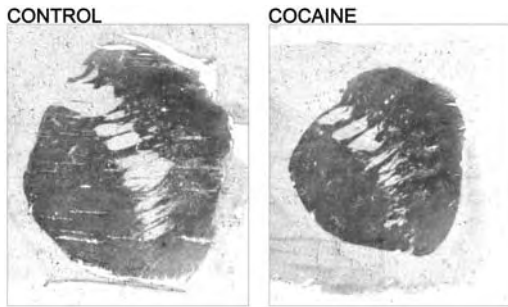

Fig. 15. Dopamine transporter binding (as revealed by [^{3}H] mazindol in the presence of desmethylimipramine, to block binding to the norepinephrine transporter) in the post-mortem striatum of a human cocaine user and normal control subject. A reduction of the dopamine transporter binding sites was found in association with cocaine use in this population (taken from Hurd and Herkenham, 1993).

with the anatomical spiral striato-nigral-striatal loop that has been documented in primates by Haber and colleagues (Haber et al., 2000; Fig. 5) in which there is a forward feed of information from the ventral striatum to the dorsal striatum.

Similar to cocaine, both the clinical and the preclinical studies indicate a profound impairment of dopaminergic markers following the use of methamphetamine. There appears to be a correlation between the methamphetamine-induced reduction of DAT measurements obtained in the living nonhuman primate and the postmortem changes in the same animal (Villemagne et al., 1998). Consistent with the recent in vivo human studies, PET analysis of nonhuman primates have demonstrated that there is recovery of the DAT binding sites following protracted methamphetamine abstinence (6 months to 1.5 years) (Harvey et al., 2000b). This finding was further confirmed by postmortem analyses of the same monkeys validating recovery of the dopamine system (e.g. DAT binding and tyrosine hydroxylase immunoreactivity) over time in a drug-free state. Moreover, there was an absence of dopamine cell loss in the dorsal and ventral tier mesencephalic group despite significant alterations of various dopamine protein markers in the terminals (Harvey et al., 2000b). Taken together, the data to date suggest that the apparent loss of in vivo DAT binding with methamphetamine use is not due to a loss of dopamine terminals in the striatum, but related instead to apparent reductions in the protein expression (and, from rat studies, in mRNA transcription). This could thus account for the recovery of DAT levels observed after protracted abstinence in previous methamphetamine users, as discussed earlier (Section 3.1.1).

In addition to the neuronal membrane DAT, the presynaptic dopamine tone is also regulated by neuronal vesicular monoamine transporters (VMAT2) that transport dopamine from the cytoplasm into synaptic storage vesicles. A reduction (cocaine, Wilson et al., 1996b; Little et al., 1999) or no change (methamphetamine, Wilson et al., 1996a; cocaine, Staley et al., 1997) of the VMAT2 binding has been found in the striatum of human stimulant users. A marked reduction of the VMAT2 immunoreactivity in the striatum (and substantia nigra) has also been observed in monkeys after methamphetamine treatment (Villemagne et al., 1998; Harvey et al., 2000a,b). Thus altered presynaptic transporters in dopaminergic neurons appears to be a characteristic feature of stimulant use.

In contrast to the marked alterations on the presynaptic DA neuronal populations in association with stimulant use, very limited alterations have been detected for DA

receptors with the postsynaptic dopaminergic markers. Thus, despite the consistent findings from in vivo PET studies, no significant changes have been found in the striatum for D_2 receptor binding (Staley et al., 1993) or mRNA (Hurd and Herkenham, 1993; Meador-Woodruff et al., 1993). However, both postmortem human and monkey studies indicate that cocaine use leads to marked alterations of D_2 receptor-related striatal populations as evidenced by a decrease of the proenkephalin mRNA expression which are primarily within the 'indirect' D_2 striatopallidal cells (Hurd and Herkenham, 1993; Daunais et al., 1997). No significant alterations have been found in postmortem studies of the D_1 receptors in relation to cocaine use (Hurd and Herkenham, 1993; Meador-Woodruff et al., 1993), but again there is a strong postmortem evidence for impairment of D_1-related striatal neuronal populations in human cocaine users. Striatal D_1 receptors predominantly express the prodynorphin gene and an upregulation of the prodynorphin mRNA expression has been documented in humans (Hurd and Herkenham, 1993) in association with cocaine use, a finding replicated in most cocaine animal models (see Hurd et al., 1999; Kreek et al., 2002). There is also evidence from nonhuman primate studies for impaired D_1 receptors in relation to cocaine use. D_1 receptor binding sites were found to be reduced in the striatum of monkeys that self-administered cocaine for a 100 days; significant changes were primarily at the posterior level of the nucleus accumbens shell and most of the adjacent dorsal striatal region at that accumbens level (Moore et al., 1998).

No changes (Meador-Woodruff et al., 1995) or an increase in striatal D_3 mRNA expression levels (Segal et al., 1997) have been reported in human cocaine abusers. The increase in the D_3 mRNA was most evident in the nucleus accumbens of cocaine overdose victims who did not present a preterminal excited delirium (Segal et al., 1997) and there was a complementary increase in the striatal D_3 binding in the cocaine fatalities (Staley and Mash, 1996). Only one study has examined the D_4 receptor with regard to stimulant abuse; no alterations were evident in the striatum of human cocaine users (Meador-Woodruff et al., 1995).

3.2. DOPAMINE SYSTEMS IN SCHIZOPHRENIA

Schizophrenia is a chronic disease characterized by, e.g. delusions, hallucinations, affective blunting, anhedonia, social withdrawal, disorganized speech, attention deficits and cognitive deficits. An extensive number of articles have been published about the dopamine hypothesis of schizophrenia (see, e.g. Sedvall and Farde, 1995; Carlsson et al., 1997; Joyce and Meador-Woodruff, 1997; Weinberger, 1997; Willner, 1997; Bennett, 1998; Laruelle, 1998; Baumeister and Francis, 2002), so below we provide only a brief review of the dopaminergic alterations that have been detected postmortem in association with this disorder.

Two main lines of evidence point to the involvement of dopamine receptors in the etiology of schizophrenia. First, all known antipsychotic medications share the ability to block D_2 dopamine receptors (Seeman et al., 1975; Farde et al., 1992; Kasper et al., 1999). Secondly, several studies have reported a subcortical hyperdopaminergic state in schizophrenia indicative of a dysregulation of dopamine in schizophrenia (Laruelle et al., 1999).

One obstacle when studying dopamine receptors in schizophrenic subjects, is that most schizophrenics have been treated with antipsychotic agents, all of which block D_2 and D_3 receptors, and many also block D_4 dopamine receptors. Thus, it can be difficult to dissociate effects specific to the disease from the effects of disease treatment. This is an

important issue since antipsychotic medication has, for example, been shown to increase the density of D_2 dopamine receptors (Joyce, 2001). One study also indicated that the slight increase detected for the D_1 dopamine receptor in the prefrontal and cingulate cortices of schizophrenic subjects was primarily related to their neuroleptic medication (Knable et al., 1996). The following paragraphs review current knowledge regarding dopaminergic alterations in schizophrenic subjects, but, as stated, some caution should be taken in regard to the influence of drug treatment.

Schizophrenic subjects generally have impaired working memory and cortical D_1 receptors have been shown to be important for cognitive performance (see, e.g. Goldman-Rakic, 1998). A postmortem examination of the densities of D_1 dopamine receptors has not consistently been found to be altered in schizophrenia (Seeman and Niznik, 1990). However, in a recent study elevated D_1 dopamine receptor binding (using [^{3}H]SCH23390) was found in the medial and inferior cortex and superior parietal cortex of schizophrenic patients as compared to controls (Domyo et al., 2001). The increases were also seen in the cerebral cortices of schizophrenic subjects off-drug for more than 40 days before death (Domyo et al., 2001). Another intriguing recent finding is that the D_1 dopamine receptor-interacting protein, calcyon, is elevated in the dorsolateral prefrontal cortex of patients with schizophrenia, an effect not mimicked by the D_2 dopamine receptor-interacting proteins (filamin-A and spinophilin) (Koh et al., 2003).

In regard to the D_2 receptors, a large number of studies have provided evidence of elevated densities of this dopamine receptor subtype in the striatum of schizophrenic subjects (see e.g. Seeman and Niznik, 1990). It has been argued that this elevation is due to treatment with antipsychotic agents, but studies have also shown elevations in drug-naïve schizophrenic brains (Joyce et al., 1988; Seeman and Niznik, 1990). In contrast to the documented increase of the striatal D_2 receptor subtype in schizophrenia, there is a report that subjects who experienced tardive dyskinesia have reduced D_2 dopamine receptors in striatal regions, but increased in the pallidum, an area of the brain particularly implicated in the production of dyskinesias (Reynolds et al., 1992). Cortical D_2-related abnormalities have also been observed in relation to schizophrenia. For example, the laminar distribution of D_2 dopamine receptors was found to be disrupted in the temporal cortex of schizophrenic subjects (Goldsmith et al., 1997). These individuals had reduced concentrations of D_2 dopamine receptors in the supragranular layers and elevated concentrations of D_2 dopamine receptors in the granular layer in isocortical regions of the temporal lobe (Goldsmith et al., 1997).

The fact that the D_3 and D_4 receptors have a strong anatomical localization to limbic and cortical brain regions (see Section 2.3) suggested their involvement in schizophrenia. However, only few postmortem studies have reported alterations in these dopamine receptor subtypes in association with this disorder. Meador-Woodruff and colleagues documented that there was a significant reduction of the D_3 and D_4 receptor mRNA levels in the orbitofrontal cortex of schizophrenic subjects, but no significant alterations in the striatum (Meador-Woodruff et al., 1997). The reduced cortical expression of the D_3 receptor mRNA in schizophrenics (Schmauss et al., 1993) has been suggested to be due to an abnormal splicing of D_3 pre-mRNA in these subjects (Schmauss, 1996). Opposite to the D_3 cortical alterations, there is evidence of increased D_3 receptor binding sites in the ventral striatum and ventral forebrain areas of schizophrenic subjects with no antipsychotic drug medication for at least a month prior to death (Gurevich et al., 1997b). Normal D_3 levels was found in those individuals with more recent neuroleptic treatment (less than 72 h prior to death) (Gurevich et al., 1997b). A three-fold elevation of

dopamine D_4 dopamine receptor-like sites has also been reported in the putamen from schizophrenic patients using [^{3}H]nemonapride (Seeman et al., 1995). However, this observation has not been replicated in other studies (Reynolds and Mason, 1994), and the specificity of the finding to the D_4 dopamine receptor has been questioned (Seeman and Van Tol, 1995).

In addition to changes in dopamine receptors, other dopaminergic impairments have been documented in association with schizophrenia. For example, there is abnormal density of TH varicosities levels in the hippocampus of schizophrenics as compared to matched controls (Benes and Todtenkopf, 1999). The average density of varicosities in the pyramidal cells and in the neuropil was found to be 30–35% lower in CA2, but not in other parts of the hippocampus of the schizophrenic subjects. Young patients showed a higher (50%) reduction on nonpyramidal cells in CA2 (Benes and Todtenkopf, 1999). A laminar-specific reduction in the density of TH-immunoreactive axons has also been observed both in the prefrontal and entorhinal cortices of subjects with schizophrenia (Akil et al., 2000). The relative density of TH-labeled axons was decreased by over 60% in layers 3 and 6, but not in layer 1, of the entorhinal cortex in schizophrenic subjects. In the prefrontal cortex of the same subjects, labeled axon density was significantly decreased by 62% only in layer 6. These findings reveal regional- and laminar-specific alterations in TH-immunoreactive axons that appear to be specific to the pathophysiology of schizophrenia (Akil et al., 2000).

3.3. DOPAMINE SYSTEMS IN AFFECTIVE DISORDERS

In contrast to elevation of DA systems that have been linked to increased reward and hyperactivity, reduced dopaminergic transmission has been proposed as one candidate for the neuropathophysiology of affective disorders. In vivo imaging studies carried out with ^{18}F-DOPA, as an index of the presynaptic dopamine function, have revealed reduced DOPA levels in the caudate nucleus of depressed subjects with affective blunting and psychomotor retardation (Martinot et al., 2001). Reduced brain dopamine has also been implicated in a clinical study in which the brain's turnover of dopamine was shown to have a significant correlation to the patients' clinical status (Lambert et al., 2000). Measurements of D_2 availability using radioligands (e.g. idobenzamide) that should compete with endogenous dopamine and provide an index of endogenous dopamine levels have revealed either an increase in binding, which would be interpreted as a reduced endogenous dopamine release (D'Haenen and Bossuyt, 1994; Shah et al., 1997) or no change (Ebert et al., 1996; Klimke et al., 1999; Parsey et al., 2001) in the striatum of depressed subjects. Differences in the clinical population and/or experimental design of the SPECT studies might account for some of the discrepancies related to D_2 receptor alterations in depressed subjects. In one report in which there was no overall differences in the D_2 receptor availability between depressed and control subjects, iodobenzamide binding was found to be increased in the patients with psychomotor retardation (Ebert et al., 1996). Moreover, antidepressant therapy led to a decrease iodobenzamide binding in subjects who improved with medication, but the dopamine D_2 receptor binding was unaltered in the non-responders (Ebert et al., 1996). It thus appears that the in vivo striatal dopamine D_2 receptor binding is not changed in depression, but is affected by psychomotor activity. An increase in D_2 dopamine receptor binding (also as revealed using iodobenzamide) in the striatum and anterior cingulate gyrus was also observed in subjects who responded to treatment with serotonin reuptake inhibitors, but not in

nonresponders (Larisch et al., 1997). In addition, the increase in D_2 dopamine receptor binding correlated significantly with the clinical recovery from depression (Larisch et al., 1997). Thus, improvement in mood state in relation to serotonin-directed antidepressant treatment could influence dopaminergic system. However, it cannot be ignored that these dopaminergic changes might relate more to enhanced psychomotor function accompanied with the positive response to antidepressant medication.

The postmortem studies have also observed impaired dopaminergic systems in relation to depression disorders. For example, D_2 dopamine receptors (visualized by [^{125}I]epidepride binding) were found to be higher in the basal, central and lateral amygdala in subjects with major depression as compared with controls (Klimek et al., 2002). There was, however, no difference in the density of D_1 dopamine receptors (by [^{3}H]SCH 23390 binding) in these same subjects (Klimek et al., 2002). In contrast to the significant alteration of D_1 dopamine receptor-interacting protein dysfunction in schizophrenics, there was no significant alteration of the cortical D_1 (or D_2) binding proteins in brains of subjects diagnosed with major depression (Koh et al., 2003).

In regard to presynaptic dopaminergic terminals in affective disorders, decreased DAT binding has been observed in the dorsal striatum of patients with major depression episodes (Meyer et al., 2001) and in untreated symptomatic depressed individuals with seasonal affective disorder (Neumeister et al., 2001). However, there is also a report of increased DAT levels in depressed subjects (Laasonen-Balk et al., 1999). This postmortem study is consistent with in vivo investigations which have found that the DAT (as studied using [^{125}I]RTI 55 binding) is significantly lower in the basal and central amygdala nuclei of depressed subjects (Klimek et al., 2002).

The postmortem investigations have failed to reveal any alterations on the binding or affinity of dopamine D_1 or D_2 receptors in the striatum of depressed subjects (Bowden et al., 1997; Allard and Norlen, 2001). However, there is a documented increase in the dopamine receptors in antidepressant-treated suicide victims, but these subjects had also received neuroleptic treatment (Bowden et al., 1997).

There is very limited information available thus far regarding the other DA receptor subtypes and affective disorders.

4. CONCLUSIONS

As reviewed in the present chapter, considerable progress has been made during the past twenty years regarding the anatomical organization of the dopamine system in the living and postmortem human brain. Despite this knowledge there are still vast gaps in our understanding about the specific role of discrete dopaminergic neural systems in neuropsychiatric disorders. One important issue is the degree to which neural alterations relate to the underlying disease state versus reflective of a response to other influences, e.g. treatment medication, exposure to other psychoactive drugs (illicit drugs or other substances such as cigarettes and alcohol) or environmental factors. Although animal studies can help to identify specific drug-related neural changes, more thorough characterization of the study participants (drug history, medical history, etc.) are needed in order to better understand the relevance of the specific neural changes to a particular disease. It is also apparent that a number of neuropsychiatric disorders are quite heterogenous with different subtypes, clinical spectrums, and potentially different underlying neurobiology at different stages of the disease. Thus, greater insight into

apparent dopaminergic involvement in these disorders will be obtained if specific subtypes (or clinical symptoms) are differentially examined instead of grouped together as one disease.

Another aspect of the research questions that have begun to be addressed and will receive even more attention in upcoming years is the relationship between genetic heterogeneity and the state or responsiveness of the neural systems examined. It is known that genetics can be critical for the development of various neuropsychiatric disease and recent studies indicate that genotype and individual variability can relate to the course of the disease and even to treatment responsiveness. For example, it has recently been shown that in vivo brain metabolic response and clinical response to the atypical antipsychotic agent, clozapine, are related to the dopamine D_1 receptor genotype (Potkin et al., 2003). Such findings substantiate the need for future studies to evaluate more thoroughly differences in dopaminergic markers in relation with the subject's genotype.

The above issues also relate very strongly to patient management since genotype can potentially influence dopamine receptor number and function which are relevant for a patient's response to antipsychotic treatment. In vivo PET and SPECT studies have documented that dopamine D_2 receptor occupancy as induced by haloperidol and other typical antipsychotics predicts not only the antipsychotic clinical response, but also drug-induced extrapyramidal side effects and akathisia (Farde et al., 1992; Nordström et al., 1993; Kapur et al., 2000). Thus, knowledge about in vivo D_2 receptors can provide a useful means of determining the correct clinical dose for schizophrenic patients with minimizing undesired side effects. However, D_2 receptor occupancy cannot alone account for all the clinical properties of antipsychotic agents considering that effective atypical medications have relatively low in vivo D_2 occupancy (Nordström et al., 1995; Talvik et al., 2001; Nyberg et al., 2002). It has long been hypothesized that a greater mesolimbic versus striatal activity accounted for the beneficial effects of atypical as compared to typical antipsychotics which are associated with higher extrapyramidal side effects (Andén, 1972; Andén and Stock, 1973). PET evaluation of human subjects have, however, now determined that there is no limbic regional selectivity in the receptor occupancy of atypical antispsychotics (Talvik et al., 2001; Nyberg et al., 2002). Clearly more investigations are required to determine what neurobiological factors are critical to consider when trying to develop effective medications which have minimal side effects.

There are still also gaps of knowledge regarding the correlation between documented in vivo neurochemical changes and symptomatology. Although there is evidence for such correlations, the data to date is not equivocal. For example, as already discussed, impaired presynaptic dopamine markers in human methamphetamine users recover following drug abstinence and is directly correlated with the dose and years of methamphetamine use (Volkow et al., 2001a). However, recovery of these presynaptic markers is not associated with a complementary improvement of motor function or cognitive skills. It is evident that a one transmitter or one protein hypothesis is unlikely to account for the vast array of symptoms associated with most psychiatric and drug addiction disorders. The ability to study multiple neurochemical systems in the same subject is a future challenge.

The focus of most human studies to date has been on the dopamine D_2 receptor and to some extent DATs in regard to neuropsychiatric disorders. It is important to emphasize that improved understanding of the in vivo dopaminergic system in disease and normal subjects is dependent on the development of specific ligands to characterize other dopaminergic markers, such as the D_3, D_4 and D_5 receptors, that have a much more

discrete anatomical organization and may contribute to the underlying neurobiology of certain neuropsychiatric disorders.

Overall, the next twenty years hold great potential for us to dissociate neural markers that underlie trait versus state which is essential for a comprehensive understanding of dopamine's involvement in specific neuropsychiatric disorders.

5. ACKNOWLEDGMENTS

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